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(72) Inventor; and		<b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> <i>With an indication in relation to a deposited microorganism furnished under Rule 13bis separately from the description.</i> <i>Date of receipt by the International Bureau:</i> <b>20 August 1996 (20.08.96)</b>	
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(88) Date of publication of the international search report: 15 May 1997 (15.05.97)			

**(54) Title: INHIBITION OF GENE EXPRESSION**

**(57) Abstract**

A method of inhibiting gene expression is described. The method, which affects enzymatic activity in a plant, comprises expressing in a plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; and wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

# INTERNATIONAL SEARCH REPORT

Intern. Application No.  
PCT/EP 96/03053

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 C12N15/82 C12N15/79 C12N15/54 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 90 12084 A (DNA PLANT TECHN CORP) 18 October 1990 see page 9, line 17 see page 11, line 25 - page 12, line 11	1-7
X	WO 92 13090 A (GEN HOSPITAL CORP) 6 August 1992 see the whole document	1,4-6
X	WO 92 14827 A (INST GENBIOLOGISCHE FORSCHUNG) 3 September 1992 see the whole document	19
A		1-10, 15-18, 20-22

-/-

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex

### \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "I" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle of the invention underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

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# INTERNATIONAL SEARCH REPORT

Intern. Appl. No.  
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## C(Conventions) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 92 11375 A (AMYLOGENE HB) 9 July 1992 see the whole document	19 1-10, 15-18, 20-22
P,X	WO 95 26407 A (NAT STARCH CHEM INVEST ;COOKE DAVID (GB); GIDLEY MICHAEL JOHN (GB)) 5 October 1995 see page 15 - page 16	19
A	PROCEEDINGS OF AN INTERNATIONAL SYMPOSIUM ON PLANT POLYMERIC CARBOHYDRATES, no. 134, 1 July 1992, pages 33-39, XP002014045 WILLMITZER L ET AL: "STARCH SYNTHESIS IN TRANSGENIC PLANTS" see page 38	2
A	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, no. 25, 5 September 1993, pages 19084-19091, XP002014043 MIZUNO K ET AL: "ALTERATION OF THE STRUCTURAL PROPERTIES OF STARCH COMPONENTS BY THE LACK OF AN ISOFORM OF STARCH BRANCHING ENZYME IN RICE SEEDS" see the whole document	2
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A	THEORETICAL AND APPLIED GENETICS, vol. 88, 1994, pages 369-375, XP002021173 FLIPSE, E., ET AL.: "Expression of a wild-type G6SS gene introduced into and amylose-free potato mutant by Agrobacterium tumefaciens and the inheritance of the inserts at the microsporic level" see page 374, left-hand column, paragraph 3 - right-hand column	1-3

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# INTERNATIONAL SEARCH REPORT

Intern. Application No.  
PCT/EP 95/03053

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MOLECULAR AND GENERAL GENETICS, vol. 246, no. 6, 20 March 1995, pages 745-755, XP000571429 KUIPERS A G J ET AL: "FACTORS AFFECTING THE INHIBITION BY ANTISENSE RNA OF GRANULE-BOUND STARCH SYNTHASE GENE EXPRESSION IN POTATO" see the whole document	1-3
A	THEORETICAL AND APPLIED GENETICS, vol. 86, 1993, pages 665-672, XP002021375 SHIMADA, H., ET AL.: "Antisense regulation of the rice waxy gene expression using a PCR-amplified fragment of rice genome reduces the amylose content in grain starch" see page 671, right-hand column, paragraph 2	1.2
A	WO 92 15680 A (UNIV TEXAS) 17 September 1992 see page 5, line 14 - page 8, line 10	1
A	CHEMICAL ABSTRACTS, vol. 121, no. 9, 29 August 1994 Columbus, Ohio, US; abstract no. 101242. SHIMADA, H., ET AL.: "Molecular cloning of cDNA and gene for rice starch branching enzyme and its use for breeding" XP002021261 see abstract & JP 06 098 656 A (MITSUI) 12 April 1994	9
A	PLANT PHYSIOLOGY, vol. 107, March 1995, pages 679-685, XP002021174 MATZKE, M.A., ET AL.: "How and why do plants inactivate homologous (trans)genes?" see the whole document	1-10, 15-22
A	WO 94 09144 A (ZENECA LTD) 28 April 1994 see page 10, line 1-18	15.22
A	WO 94 11520 A (ZENECA LTD ; KEELING PETER LEWIS (GB)) 26 May 1994 see claims 6,13	15.22
A	US 4 740 463 A (WEINBERG ROBERT A ET AL) 26 April 1988 see column 8 - column 9	15.22

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 96/03053

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos. because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos. because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos. because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International Application, as follows:

see continuation-sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort involving an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims, i.e. covered by claims Nos.

1-10, 15, 20, 21, 22 (all completely) and 16-19 (all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 96/ 03053

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1. Claims: 1-10,15,20,21,22 (all completely) and 16-19 (all partially)  
Method for affecting enzyme/protein activity using sense intron  
sequences, particularly affecting SBE, amylopectin levels or starch  
composition.

2. Claims: 11-14 (all completely) and 16-19 (all partially)  
Method for tissue specific expression of transgenes using the SBE promoter  
of Seq. ID.14.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. Pat. Appl. No. 95/00053

PCT/EP 95/00053

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Information on patent family members

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VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian  
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patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT,  
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(54) Title: INHIBITION OF GENE EXPRESSION

(57) Abstract

A method of inhibiting gene expression is described. The method, which affects enzymatic activity in a plant, comprises expressing in a plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; and wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

## INHIBITION OF GENE EXPRESSION

The present invention relates to a method of inhibiting gene expression, particularly inhibiting gene expression in a plant. The present invention also relates to a  
5 nucleotide sequence useful in the method. In addition, the present invention relates to a promoter that is useful for expressing the nucleotide sequence.

Starch is one of the main storage carbohydrates in plants, especially higher plants. The structure of starch consists of amylose and amylopectin. Amylose consists  
10 essentially of straight chains of  $\alpha$ -1-4-linked glycosyl residues. Amylopectin comprises chains of  $\alpha$ -1-4-linked glycosyl residues with some  $\alpha$ -1-6 branches. The branched nature of amylopectin is accomplished by the action of *inter alia* an enzyme commonly known as the starch branching enzyme ("SBE"). SBE catalyses the formation of branch points in the amylopectin molecule by adding  $\alpha$ -1,4 glucans  
15 through  $\alpha$ -1,6-glucosidic branching linkages. The biosynthesis of amylose and amylopectin is schematically shown in Figure 1, whereas the  $\alpha$ -1-4-links and the  $\alpha$ -1-6 links are shown in Figure 2.

It is known that starch is an important raw material. Starch is widely used in the  
20 food, paper, and chemical industries. However, a large fraction of the starches used in these industrial applications are post-harvest modified by chemical, physical or enzymatic methods in order to obtain starches with certain required functional properties.

Within the past few years it has become desirable to make genetically modified plants which could be capable of producing modified starches which could be the same as the post-harvest modified starches. It is also known that it may be possible to prepare such genetically modified plants by expression of antisense nucleotide coding sequences. In this regard, June Bourque provides a detailed summary of antisense  
30 strategies for the genetic manipulations in plants (Bourque 1995 Plant Science 105 pp 125-149).

Whilst it is known that enzymatic activity can be affected by expression of particular nucleotide sequences (for example see the teachings of Finnegan and McElroy [1994] Biotechnology 12 883-888; and Matzke and Matzke [1995] TIG 11 1-3) there is still a need for a method that can more reliably and/or more efficiently and/or more specifically affect enzymatic activity.

According to a first aspect of the present invention there is provided a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence partially or completely codes (is) an intron in a sense orientation; and wherein the nucleotide sequence does not contain a sequence that is a sense exon sequence normally associated with the intron.

According to a second aspect of the present invention there is provided a method of affecting enzymatic activity in a starch producing organism (or a cell, a tissue or an organ thereof) comprising expressing in the starch producing organism (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; and wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed.

According to a third aspect of the present invention there is provided a sequence comprising the nucleotide sequence shown as any one of SEQ.I.D. No. 1 to SEQ.I.D. No. 13 or a variant, derivative or homologue thereof.

According to a fourth aspect of the present invention there is provided a promoter comprising the sequence shown as SEQ.I.D. No. 14 or a variant, derivative or homologue thereof.

According to a fifth aspect of the present invention there is provided a construct capable of comprising or expressing the present invention.

5 According to a sixth aspect of the present invention there is provided a vector comprising or expressing the present invention.

According to a seventh aspect of the present invention there is provided a cell, tissue or organ comprising or expressing the present invention.

10 According to an eighth aspect of the present invention there is provided a transgenic starch producing organism comprising or expressing the present invention. According to a ninth aspect of the present invention there is provided a starch obtained from the present invention.

15 According to a tenth aspect of the present invention there is provided pBEA11 (NCIMB 40754). According to an eleventh aspect of the present invention there is provided a sense nucleotide sequence that is obtainable from  $\lambda$ -SBE 3.2 (NCIMB 40751) or  $\lambda$ -SBE 3.4 (NCIMB 40752) or a variant, derivative or homologue thereof.

20 A key advantage of the present invention is that it provides a method for preparing modified starches that is not dependent on the need for post-harvest modification of starches. Thus the method of the present invention obviates the need for the use of hazardous chemicals that are normally used in the post-harvest modification of starches.

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In addition, the present invention provides *inter alia* genetically modified plants which are capable of producing modified and/or novel and/or improved starches whose properties would satisfy various industrial requirements.

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Thus, the present invention provides a method of preparing tailor-made starches in plants which could replace the post-harvest modified starches.

Also, the present invention provides a method that enables modified starches to be prepared by a method that can have a more beneficial effect on the environment than the known post-harvest modification methods which are dependent on the use of hazardous chemicals and large quantities of energy.

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An other key advantage of the present invention is that it provides a method that may more reliably and/or more efficiently and/or more specifically affect enzymatic activity when compared to the known methods of affecting enzymatic activity. With regard to this advantage of the present invention it is to be noted that there is some degree of homology between coding regions of SBEs. However, there is little or no homology with the intron sequences of SBEs. Thus, sense intron expression provides a mechanism to affect selectively the expression of a particular SBE. This advantageous aspect could be used, for example, to reduce or eliminate a particular SBE enzyme and replace that enzyme with another enzyme which can be another branching enzyme or even a recombinant version of the affected enzyme or even a hybrid enzyme which could for example comprise part of a SBE enzyme from one source and at least a part of another SBE enzyme from another source. This particular feature of the present invention is covered by the combination aspect of the present invention which is discussed in more detail later.

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Thus the present invention provides a mechanism for selectively affecting SBE activity. This is in contrast to the prior art methods which are dependent on the use of for example antisense exon expression whereby it would not be possible to introduce new SBE activity without affecting that activity as well.

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Preferably with the first aspect of the present invention starch branching enzyme activity is affected and/or wherein the levels of amylopectin are affected and/or the composition of starch is changed.

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Preferably with the first or second aspect of the present invention the nucleotide sequence does not contain a sequence that is sense to an exon sequence.

Preferably with the fourth aspect of the present invention the promoter is in combination with a gene of interest ("GOI").

Preferably the enzymatic activity is reduced or eliminated.

5

Preferably the nucleotide sequence codes for at least substantially all of at least one intron in a sense orientation.

10

Preferably the nucleotide sequence codes, partially or completely, for two or more introns and wherein each intron is in a sense orientation.

Preferably the nucleotide sequence comprises at least 350 nucleotides (e.g. 350 bp), more preferably at least 500 nucleotides (e.g. 500 bp).

15

Preferably the nucleotide sequence comprises the sequence shown as any one of SEQ. I.D. No. 1 to SEQ. I.D. No. 13 or a variant, derivative or homologue thereof, including combinations thereof.

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Preferably the nucleotide sequence is expressed by a promoter having a sequence shown as SEQ. I.D. No. 14 or a variant, derivative or homologue thereof.

Preferably the transgenic starch producing organism is a plant.

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A preferred aspect of the present invention therefore relates to a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that

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is sense to an exon sequence normally associated with the intron; and wherein the branching enzyme activity is affected and/or the levels of amylose are affected and/or the composition of starch is changed.

A more preferred aspect of the present invention therefore relates to a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation: wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron: wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed: and wherein the nucleotide sequence comprises the sequence shown as any one of SEQ.I.D. No. 1 to SEQ.I.D. No. 13 or a variant, derivative or homologue thereof, including combinations thereof.

The term "nucleotide" in relation to the present invention includes DNA and RNA. Preferably it means DNA, more preferably DNA prepared by use of recombinant DNA techniques.

The term "intron" is used in its normal sense as meaning a segment of nucleotides, usually DNA, that does not encode part or all of an expressed protein or enzyme.

The term "exon" is used in its normal sense as meaning a segment of nucleotides, usually DNA, encoding part or all of an expressed protein or enzyme.

Thus, the term "intron" refers to gene regions that are transcribed into RNA molecules, but which are spliced out of the RNA before the RNA is translated into a protein. In contrast, the term "exon" refers to gene regions that are transcribed into RNA and subsequently translated into proteins.

The terms "variant" or "homologue" or "fragment" in relation to the nucleotide sequence of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the respective nucleotide sequence providing the resultant nucleotide sequence can affect enzyme activity in a plant, or cell or tissue thereof, preferably wherein the resultant nucleotide sequence has at least the same effect as any one of

the sense sequences shown as SEQ.I.D. No.s 1-13. In particular, the term "homologue" covers homology with respect to similarity of structure and/or similarity of function providing the resultant nucleotide sequence has the ability to affect enzymatic activity in accordance with the present invention. With respect to sequence homology (i.e. similarity), preferably there is more than 80% homology, more preferably at least 85% homology, more preferably at least 90% homology, even more preferably at least 95% homology, more preferably at least 98% homology. The above terms are also synonymous with allelic variations of the sequences.

Likewise, the terms "variant" or "homologue" or "fragment" in relation to the promoter of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the respective promoter sequence providing the resultant promoter sequence allows expression of a GOI, preferably wherein the resultant promoter sequence has at least the same effect as SEQ.I.D. No. 14. In particular, the term "homologue" covers homology with respect to similarity of structure and/or similarity of function providing the resultant promoter sequence has the ability to allow for expression of a GOI, such as a nucleotide sequence according to the present invention. With respect to sequence homology (i.e. similarity), preferably there is more than 80% homology, more preferably at least 85% homology, more preferably at least 90% homology, even more preferably at least 95% homology, more preferably at least 98% homology. The above terms are also synonymous with allelic variations of the sequences.

The intron sequence of the present invention can be any one or all of the intron sequences of the present invention, including partial sequences thereof, provided that if partial sense sequences are used (i.e. sequences that are not or do not comprise any one or more of the full sequences shown as SEQ.I.D. No.1-13) the partial sequences affect enzymatic activity. Suitable examples of partial sequences include sequences that are shorter than any one of the full sense sequences shown as SEQ.I.D.No.s 1 to 13 but which comprise nucleotides that are adjacent the respective exon or exons.



With regard to the second aspect of the present invention (i.e. specifically affecting SBE activity), the nucleotide sequences of the present invention may comprise one or more sense or antisense exon sequences of the SBE gene (but not sense exon sequences naturally associated with the intron sequence), including complete or partial sequences thereof, providing the nucleotide sequences can affect SBE activity, preferably wherein the nucleotide sequences reduce or eliminate SBE activity. Preferably, the nucleotide sequence of the second aspect of the present invention does not comprise sense exon sequences.

The term "vector" includes an expression vector and a transformation vector. The term "expression vector" means a construct capable of *in vivo* or *in vitro* expression. The term "transformation vector" means a construct capable of being transferred from one species to another - such as from an *E. Coli* plasmid to a fungus or a plant cell, or from an *Agrobacterium* to a plant cell.

The term "construct" - which is synonymous with terms such as "conjugate", "cassette" and "hybrid" - in relation to the sense nucleotide sequence aspect of the present invention includes the nucleotide sequence according to the present invention directly or indirectly attached to a promoter. An example of an indirect attachment is the provision of a suitable spacer group such as an intron sequence, such as the *ShI*-intron or the ADH intron, intermediate the promoter and the nucleotide sequence of the present invention. The same is true for the term "fused" in relation to the present invention which includes direct or indirect attachment. The terms do not cover the natural combination of the wild type SBE gene when associated with the wild type SBE gene promoter in their natural environment.

The construct may even contain or express a marker which allows for the selection of the genetic construct in, for example, a plant cell into which it has been transferred. Various markers exist which may be used in, for example, plants - such as mannose. Other examples of markers include those that provide for antibiotic resistance - e.g. resistance to G418, hygromycin, bleomycin, kanamycin and gentamycin.

The construct of the present invention preferably comprises a promoter. The term "promoter" is used in the normal sense of the art, e.g. an RNA polymerase binding site in the Jacob-Monod theory of gene expression. Examples of suitable promoters are those that can direct efficient expression of the nucleotide sequence of the present invention and/or in a specific type of cell. Some examples of tissue specific promoters are disclosed in WO 92/11375.

The promoter could additionally include conserved regions such as a Pribnow Box or a TATA box. The promoters may even contain other sequences to affect (such as to maintain, enhance, decrease) the levels of expression of the nucleotide sequence of the present invention. Suitable examples of such sequences include the *Sh1*-intron or an ADH intron. Other sequences include inducible elements - such as temperature, chemical, light or stress inducible elements. Also, suitable elements to enhance transcription or translation may be present. An example of the latter element is the TMV 5' leader sequence (see Sleat Gene 217 [1987] 217-225; and Dawson Plant Mol. Biol. 23 [1993] 97).

As mentioned, the construct and/or the vector of the present invention may include a transcriptional initiation region which may provide for regulated or constitutive expression. Any suitable promoter may be used for the transcriptional initiation region, such as a tissue specific promoter. In one aspect, preferably the promoter is the patatin promoter or the E35S promoter. In another aspect, preferably the promoter is the SBE promoter.

If, for example, the organism is a plant then the promoter can be one that affects expression of the nucleotide sequence in any one or more of seed, tuber, stem, sprout, root and leaf tissues, preferably tuber. By way of example, the promoter for the nucleotide sequence of the present invention can be the  $\alpha$ -Amy 1 promoter (otherwise known as the Amy 1 promoter, the Amy 637 promoter or the  $\alpha$ -Amy 637 promoter) as described in our co-pending UK patent application No. 9421292.5 filed 21 October 1994. Alternatively, the promoter for the nucleotide sequence of the present invention can be the  $\alpha$ -Amy 3 promoter (otherwise known as the Amy 3

promoter, the Amy 351 promoter or the  $\alpha$ -Amy 351 promoter) as described in our co-pending UK patent application No. 9421286.7 filed 21 October 1994.

5 The present invention also encompasses the use of a promoter to express a nucleotide sequence according to the present invention, wherein a part of the promoter is inactivated but wherein the promoter can still function as a promoter. Partial inactivation of a promoter in some instances is advantageous. In particular, with the Amy 351 promoter mentioned earlier it is possible to inactivate a part of it so that the partially inactivated promoter expresses the nucleotide sequence of the present  
10 invention in a more specific manner such as in just one specific tissue type or organ. The term "inactivated" means partial inactivation in the sense that the expression pattern of the promoter is modified but wherein the partially inactivated promoter still functions as a promoter. However, as mentioned above, the modified promoter is capable of expressing a gene coding for the enzyme of the present invention in at least one (but not all) specific tissue of the original promoter. Examples of partial  
15 inactivation include altering the folding pattern of the promoter sequence, or binding species to parts of the nucleotide sequence, so that a part of the nucleotide sequence is not recognised by, for example, RNA polymerase. Another, and preferable, way of partially inactivating the promoter is to truncate it to form fragments thereof. Another way would be to mutate at least a part of the sequence so that the RNA  
20 polymerase can not bind to that part or another part. Another modification is to mutate the binding sites for regulatory proteins for example the CreA protein known from filamentous fungi to exert carbon catabolite repression, and thus abolish the catabolite repression of the native promoter.

25 The construct and/or the vector of the present invention may include a transcriptional termination region.

30 The nucleotide according to the present invention can be expressed in combination (but not necessarily at the same time) with an additional construct. Thus the present invention also provides a combination of constructs comprising a first construct comprising the nucleotide sequence according to the present invention operatively

linked to a first promoter; and a second construct comprising a GOI operatively linked to a second promoter (which need not be the same as the first promoter). With this aspect of the present invention the combination of constructs may be present in the same vector, plasmid, cells, tissue, organ or organism. This aspect of the present invention also covers methods of expressing the same, preferably in specific cells or tissues, such as expression in just a specific cell or tissue, of an organism, typically a plant. With this aspect of the present invention the second construct does not cover the natural combination of the gene coding for an enzyme ordinarily associated with the wild type gene promoter when they are both in their natural environment.

An example of a suitable combination would be a first construct comprising the nucleotide sequence of the present invention and a promoter, such as the promoter of the present invention, and a second construct comprising a promoter, such as the promoter of the present invention, and a GOI wherein the GOI codes for another starch branching enzyme either in sense or antisense orientation.

The above comments relating to the term "construct" for the sense nucleotide aspect of the present invention are equally applicable to the term "construct" for the promoter aspect of the present invention. In this regard, the term includes the promoter according to the present invention directly or indirectly attached to a GOI.

The term "GOI" with reference to the promoter aspect of the present invention or the combination aspect of the present invention means any gene of interest, which need not necessarily code for a protein or an enzyme - as is explained later. A GOI can be any nucleotide sequence that is either foreign or natural to the organism in question, for example a plant.

Typical examples of a GOI include genes encoding for other proteins or enzymes that modify metabolic and catabolic processes. The GOI may code for an agent for introducing or increasing pathogen resistance.

The GOI may even be an antisense construct for modifying the expression of natural transcripts present in the relevant tissues. An example of such a GOI is the nucleotide sequence according to the present invention.

5 The GOI may even code for a protein that is non-natural to the host organism - e.g. a plant. The GOI may code for a compound that is of benefit to animals or humans. For example, the GOI could code for a pharmaceutically active protein or enzyme such as any one of the therapeutic compounds insulin, interferon, human serum albumin, human growth factor and blood clotting factors. The GOI may even code  
10 for a protein giving additional nutritional value to a food or feed or crop. Typical examples include plant proteins that can inhibit the formation of anti-nutritive factors and plant proteins that have a more desirable amino acid composition (e.g. a higher lysine content than a non-transgenic plant). The GOI may even code for an enzyme that can be used in food processing such as xylanases and  $\alpha$ -galactosidase. The GOI  
15 can be a gene encoding for any one of a pest toxin, an antisense transcript such as that for  $\alpha$ -amylase, a protease or a glucanase. Alternatively, the GOI can be a nucleotide sequence according to the present invention.

The GOI can be the nucleotide sequence coding for the arabinofuranosidase enzyme  
20 which is the subject of our co-pending UK patent application 9505479.7. The GOI can be the nucleotide sequence coding for the glucanase enzyme which is the subject of our co-pending UK patent application 9505475.5. The GOI can be the nucleotide sequence coding for the  $\alpha$ -amylase enzyme which is the subject of our co-pending UK patent application 9413439.2. The GOI can be the nucleotide sequence coding for  
25 the  $\alpha$ -amylase enzyme which is the subject of our co-pending UK patent application 9421290.9. The GOI can be any of the nucleotide sequences coding for the  $\alpha$ -glucan lyase enzyme which are described in our co-pending PCT patent application PCT/EP94/03397.

30 In one aspect the GOI can even be a nucleotide sequence according to the present invention but when operatively linked to a different promoter.

The GOI could include a sequence that codes for one or more of a xylanase, an arabinase, an acetyl esterase, a rhamnogalacturonase, a glucanase, a pectinase, a branching enzyme or another carbohydrate modifying enzyme or proteinase. Alternatively, the GOI may be a sequence that is antisense to any of those sequences.

5

As mentioned above, the present invention provides a mechanism for selectively affecting a particular enzymatic activity.

In an important application of the present invention it is now possible to reduce or eliminate expression of a genomic nucleotide sequence coding for a genomic protein or enzyme by expressing a sense intron construct for that particular genomic protein or enzyme and (e.g. at the same time) expressing a recombinant version of that enzyme or protein - in other words the GOI is a recombinant nucleotide sequence coding for the genomic enzyme or protein. This application allows expression of desired recombinant enzymes and proteins in the absence of (or reduced levels of) respective genomic enzymes and proteins. Thus the desired recombinant enzymes and proteins can be easily separated and purified from the host organism. This particular aspect of the present invention is very advantageous over the prior art methods which, for example, rely on the use of anti-sense exon expression which methods also affect expression of the recombinant enzyme.

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Thus, a further aspect of the present invention relates to a method of expressing a recombinant protein or enzyme in a host organism comprising expressing a nucleotide sequence coding for the recombinant protein or enzyme; and expressing a further nucleotide sequence wherein the further nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the intron is an intron normally associated with the genomic gene encoding a protein or an enzyme corresponding to the recombinant protein or enzyme; and wherein the further nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron. Additional aspects cover the combination of those nucleotide sequences including their incorporation in constructs, vectors, cells, tissues and transgenic organisms.

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Therefore the present invention also relates to a combination of nucleotide sequences comprising a first nucleotide sequence coding for a recombinant enzyme; and a second nucleotide sequence which corresponds to an intron in a sense orientation; wherein the intron is an intron that is associated with a genomic gene encoding the enzyme corresponding to the recombinant enzyme; and wherein the second nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

The GOI may even code for one or more introns but in an antisense orientation, such as any one or more of the antisense intron sequences presented in the attached sequence listings. For example, the present invention also covers the expression of for example a sense intron (e.g. SEQ.I.D.No. 1) in combination with for example an antisense sense intron which preferably is not complementary to the sense intron sequence (e.g. SEQ.I.D.No. 16).

The terms "cell", "tissue" and "organ" include cell, tissue and organ *per se* and when within an organism.

The term "organism" in relation to the present invention includes any organism that could comprise the nucleotide sequence according to the present invention and/or wherein the nucleotide sequence according to the present invention can be expressed when present in the organism. Preferably the organism is a starch producing organism such as any one of a plant, algae, fungi, yeast and bacteria, as well as cell lines thereof. Preferably the organism is a plant.

The term "starch producing organism" includes any organism that can biosynthesise starch. Preferably, the starch producing organism is a plant.

The term "plant" as used herein includes any suitable angiosperm, gymnosperm, monocotyledon and dicotyledon. Typical examples of suitable plants include vegetables such as potatoes; cereals such as wheat, maize, and barley; fruit; trees; flowers; and other plant crops. Preferably, the term means "potato".

The term "transgenic organism" in relation to the present invention includes any organism that comprises the nucleotide sequence according to the present invention and/or products obtained therefrom, and/or wherein the nucleotide sequence according to the present invention can be expressed within the organism. Preferably the nucleotide sequence of the present invention is incorporated in the genome of the organism. Preferably the transgenic organism is a plant, more preferably a potato.

To prepare the host organism one can use prokaryotic or eukaryotic organisms. Examples of suitable prokaryotic hosts include *E. coli* and *Bacillus subtilis*. Teachings on the transformation of prokaryotic hosts is well documented in the art, for example see Sambrook *et al* (Sambrook *et al.* in *Molecular Cloning: A Laboratory Manual*, 2nd edition, 1989, Cold Spring Harbor Laboratory Press).

Even though the enzyme according to the present invention and the nucleotide sequence coding for same are not disclosed in EP-B-0470145 and CA-A-2006454, those two documents do provide some useful background commentary on the types of techniques that may be employed to prepare transgenic plants according to the present invention. Some of these background teachings are now included in the following commentary.

The basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material.

Several techniques exist for inserting the genetic information, the two main principles being direct introduction of the genetic information and introduction of the genetic information by use of a vector system. A review of the general techniques may be found in articles by Potrykus (*Annu Rev Plant Physiol Plant Mol Biol* [1991] 42:205-225) and Christou (*Agro-Food-Industry Hi-Tech* March/April 1994 17-27).

Thus, in one aspect, the present invention relates to a vector system which carries a nucleotide sequence or construct according to the present invention and which is



capable of introducing the nucleotide sequence or construct into the genome of an organism, such as a plant.

5 The vector system may comprise one vector, but it can comprise two vectors. In the case of two vectors, the vector system is normally referred to as a binary vector system. Binary vector systems are described in further detail in Gynheung An *et al.* (1980), Binary Vectors, *Plant Molecular Biology Manual A3*, 1-19.

10 One extensively employed system for transformation of plant cells with a given promoter or nucleotide sequence or construct is based on the use of a Ti plasmid from *Agrobacterium tumefaciens* or a Ri plasmid from *Agrobacterium rhizogenes* An *et al.* (1986), *Plant Physiol.* 81, 301-305 and Butcher D.N. *et al.* (1980), *Tissue Culture Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208. Several different Ti and Ri plasmids have been constructed which are suitable for the  
15 construction of the plant or plant cell constructs described above. A non-limiting example of such a Ti plasmid is pGV3850.

The nucleotide sequence or construct of the present invention should preferably be inserted into the Ti-plasmid between the terminal sequences of the T-DNA or adjacent  
20 a T-DNA sequence so as to avoid disruption of the sequences immediately surrounding the T-DNA borders, as at least one of these regions appears to be essential for insertion of modified T-DNA into the plant genome.

As will be understood from the above explanation, if the organism is a plant the  
25 vector system of the present invention is preferably one which contains the sequences necessary to infect the plant (e.g. the *vir* region) and at least one border part of a T-DNA sequence, the border part being located on the same vector as the genetic construct.

30 Furthermore, the vector system is preferably an *Agrobacterium tumefaciens* Ti-plasmid or an *Agrobacterium rhizogenes* Ri-plasmid or a derivative thereof. As these plasmids are well-known and widely employed in the construction of transgenic

plants, many vector systems exist which are based on these plasmids or derivatives thereof.

In the construction of a transgenic plant the nucleotide sequence or construct of the present invention may be first constructed in a microorganism in which the vector can replicate and which is easy to manipulate before insertion into the plant. An example of a useful microorganism is *E. coli*, but other microorganisms having the above properties may be used. When a vector of a vector system as defined above has been constructed in *E. coli*, it is transferred, if necessary, into a suitable *Agrobacterium* strain, e.g. *Agrobacterium tumefaciens*. The Ti-plasmid harbouring the nucleotide sequence or construct of the present invention is thus preferably transferred into a suitable *Agrobacterium* strain, e.g. *A. tumefaciens*, so as to obtain an *Agrobacterium* cell harbouring the promoter or nucleotide sequence or construct of the present invention, which DNA is subsequently transferred into the plant cell to be modified.

If, for example, for the transformation the Ti- or Ri-plasmid of the plant cells is used, at least the right boundary and often however the right and the left boundary of the Ti- and Ri-plasmid T-DNA, as flanking areas of the introduced genes, can be connected. The use of T-DNA for the transformation of plant cells has been intensively studied and is described in EP-A-120516; Hoekema, in: The Binary Plant Vector System Offset-drukkerij Kanters B.B., Alblaserdam, 1985, Chapter V; Fraley, *et al.*, Crit. Rev. Plant Sci., 4:1-46; and An *et al.*, EMBO J. (1985) 4:277-284.

Direct infection of plant tissues by *Agrobacterium* is a simple technique which has been widely employed and which is described in Butcher D.N. *et al.* (1980), *Tissue Culture Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208. For further teachings on this topic see Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27). With this technique, infection of a plant may be performed in or on a certain part or tissue of the plant, i.e. on a part of a leaf, a root, a stem or another part of the plant.

Typically, with direct infection of plant tissues by *Agrobacterium* carrying the GOI (such as the nucleotide sequence according to the present invention) and, optionally, a promoter, a plant to be infected is wounded, e.g. by cutting the plant with a razor blade or puncturing the plant with a needle or rubbing the plant with an abrasive. The wound is then inoculated with the *Agrobacterium*. The inoculated plant or plant part is then grown on a suitable culture medium and allowed to develop into mature plants.

When plant cells are constructed, these cells may be grown and maintained in accordance with well-known tissue culturing methods such as by culturing the cells in a suitable culture medium supplied with the necessary growth factors such as amino acids, plant hormones, vitamins, etc.

Regeneration of the transformed cells into genetically modified plants may be accomplished using known methods for the regeneration of plants from cell or tissue cultures, for example by selecting transformed shoots using an antibiotic and by subculturing the shoots on a medium containing the appropriate nutrients, plant hormones, etc.

Further teachings on plant transformation may be found in EP-A-0449375.

As reported in CA-A-2006454, a large amount of cloning vectors are available which contain a replication system in *E. coli* and a marker which allows a selection of the transformed cells. The vectors contain for example pBR 322, pUC series, M13 mp series, pACYC 184 etc. In this way, the nucleotide or construct of the present invention can be introduced into a suitable restriction position in the vector. The contained plasmid is then used for the transformation in *E. coli*. The *E. coli* cells are cultivated in a suitable nutrient medium and then harvested and lysed. The plasmid is then recovered. As a method of analysis there is generally used sequence analysis, restriction analysis, electrophoresis and further biochemical-molecular biological methods. After each manipulation, the used DNA sequence can be restricted and connected with the next DNA sequence. Each sequence can be cloned in the same

- or different plasmid.

After the introduction of the nucleotide sequence or construct according to the present invention in the plants the presence and/or insertion of further DNA sequences may  
5 be necessary - such as to create combination systems as outlined above (e.g. an organism comprising a combination of constructs).

The above commentary for the transformation of prokaryotic organisms and plants with the nucleotide sequence of the present invention is equally applicable for the  
10 transformation of those organisms with the promoter of the present invention.

In summation, the present invention relates to affecting enzyme activity by expressing sense intron sequences.

15 Also, the present invention relates to a promoter useful for the expression of those sense intron sequences.

The following samples have been deposited in accordance with the Budapest Treaty at the recognised depositary The National Collections of Industrial and Marine  
20 Bacteria Limited (NCIMB) at 23 St Machar Drive, Aberdeen, Scotland, AB2 1RY, United Kingdom, on 13 July 1995:

NCIMB 40754 (which refers to pBEA 11 as described herein);

25 NCIMB 40751 (which refers to  $\lambda$ -SBE 3.2 as described herein), and

NCIMB 40752 (which refers to  $\lambda$ -SBE 3.4 as described herein).

A highly preferred embodiment of the present invention therefore relates to a method  
30 of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely,

for an intron in a sense orientation: wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed; and wherein the intron nucleotide sequence is obtainable from NCIMB 40751, NCIMB 40752, or NCIMB 40754 or a variant, derivative or homologue thereof.

A more highly preferred aspect of the present invention therefore relates to a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed; wherein the nucleotide sequence comprises the sequence shown as any one of SEQ.I.D. No. 1 to SEQ.I.D. No. 13 or a variant, derivative or homologue thereof, including combinations thereof; and wherein the intron nucleotide sequence is obtainable from NCIMB 40751, NCIMB 40752, or NCIMB 40754, or a variant, derivative or homologue thereof.

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The present invention will now be described only by way of example, in which reference is made to the following attached Figures:

Figure 1, which is a schematic representation of the biosynthesis of amylose and amylopectin;

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Figure 2, which is a diagrammatic representation of the  $\alpha$ -1-4-links and the  $\alpha$ -1-6 links of amylopectin;

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Figure 3, which is a diagrammatic representation of the exon-intron structure of a genomic SBE clone;

Figure 4, which is a plasmid map of pPATA1, which is 3936 bp in size:

Figure 5, which is a plasmid map of pABE7, which is 5106 bp in size:

5 Figure 6, which is a plasmid map of pVictorIV Man, which is 7080 bp in size:

Figure 7, which is a plasmid map of pBEA11, which is 9.54 kb in size:

10 Figure 8, which shows the full genomic nucleotide sequence for SBE including the promoter, exons and introns:

Figure 9, which is a plasmid map of pVictor5a, which is 9.12 kb in size; and

15 Figure 10, which is a plasmid map of pBEP2, which is 10.32 kb in size.

Figures 1 and 2 were referred to above in the introductory description concerning starch in general. As mentioned, Figure 3 is a diagrammatic representation of the exon-intron structure of a genomic SBE clone, the sequence of which is shown in Figure 8. This clone, which has about 11.5 k base pairs, comprises 14 exons and 13  
20 introns. The introns are numbered in increasing order from the 5' end to the 3' end and correspond to SEQ.I.D.No.s 1-13, respectively. Their respective antisense intron sequences are shown as SEQ.I.D.No.s 15-27.

In more detail, Figures 3 and 8 present information on the 11468 base pairs of a  
25 potato SBE gene. The 5' region from nucleotides 1 to 2082 contain the promoter region of the SBE gene. A TATA box candidate at nucleotide 2048 to 2051 is boxed. The homology between a potato SBE cDNA clone (Poulsen & Kreiberg (1993) Plant Physiol 102: 1053-1054) and the exon DNAs begin at 2083 bp and end at 9666 bp. The homology between the cDNA and the exon DNA is indicated by nucleotides in  
30 upper case letters, while the translated amino acid sequences are shown in the single letter code below the exon DNA. Intron sequences are indicated by lower case letters.

Figure 7 is a plasmid map of pBEA7, which is 9.54 k base pairs in size. Plasmid pBEA 11 comprises the first intron sequence of the potato SBE gene. This first intron sequence, which has 1177 base pairs, is shown in Figure 3 and lies between the first exon and the second exon.

These experiments and aspects of the present invention are now discussed in more detail.

#### EXPERIMENTAL PROTOCOL

#### ISOLATION, SUBCLONING IN PLASMIDS, AND SEQUENCING OF GENOMIC SBE CLONES

Various clones containing the potato SBE gene were isolated from a Desiree potato genomic library (Clontech Laboratories Inc., Palo Alto CA, USA) using radioactively labelled potato SBE cDNA (Poulsen & Kreiberg (1993) Plant Physiol. 102:1053-1054) as probe. The fragments of the isolated  $\lambda$ -phages containing SBE DNA ( $\lambda$ SBE 3.2 - NCIMB 40751 - and  $\lambda$ SBE-3.4 - NCIMB 40752) were identified by Southern analysis and then subcloned into pBluescript II vectors (Clontech Laboratories Inc., Palo Alto CA, USA).  $\lambda$ SBE 3.2 contains a 15 kb potato DNA insert and  $\lambda$ SBE-3.4 contains a 13 kb potato DNA insert. The resultant plasmids were called pGB3, pGB11, pGB15, pGB16 and pGB25 (see discussion below). The respective inserts were then sequenced using the Pharmacia Autoread Sequencing Kit (Pharmacia, Uppsala) and a A.L.F. DNA sequencer (Pharmacia, Uppsala).

In total, a stretch of 11.5 kb of the SBE gene was sequenced. The sequence was deduced from the above-mentioned plasmids, wherein: pGB25 contains the sequences from 1 bp to 836 bp, pGB15 contains the sequences from 735 bp to 2580 bp, pGB16 contains the sequences from 2580 bp to 5093 bp, pGB11 contains the sequences from 3348 bp to 7975 bp, and pGB3 contains the sequences from 7533 bp to 11468 bp.

In more detail, pGB3 was constructed by insertion of a 4 kb *EcoRI* fragment isolated from  $\lambda$ SBE 3.2 into the *EcoRI* site of pBluescript II SK (+). pGB11 was constructed by insertion of a 4.7 kb *XhoI* fragment isolated from  $\lambda$ SBE 3.4 into the *XhoI* site of pBluescript II SK (+). pGB15 was constructed by insertion of a 1.7 kb *SpeI* fragment isolated from  $\lambda$ SBE 3.4 into the *SpeI* site of pBluescript II SK (+). pGB16 was constructed by insertion of a 2.5 kb *SpeI* fragment isolated from  $\lambda$ SBE 3.4 into the *SpeI* site of pBluescript II SK (+). For the construction of pGB25 a PCR fragment was produced with the primers

5' GGA ATT CCA GTC GCA GTC TAC ATT AC 3'

and

5' CGG GAT CCA GAG GCA TTA AGA TTT CTG G 3'

and  $\lambda$ SBE 3.4 as a template.

The PCR fragment was digested with *BamHI* and *EcoRI*, and inserted in pBluescript II SK (+) digested with the same restriction enzymes.

#### CONSTRUCTION OF PLASMID pBEA11

The SBE intron 1 was amplified by PCR using the oligonucleotides

5' CGG GAT CCA AAG AAA TTC TCG AGG TTA CAT GG 3'

and

5' CGG GAT CCG GGG TAA TTT TTA CTA ATT TCA TG 3'

and the  $\lambda$ SBE 3.4 phage containing the SBE gene as template.



The PCR product was digested with *Bam*HI and inserted in a sense orientation in the *Bam*HI site of plasmid pPATA1 (described in WO 94/24292) between the patatin promoter and the 35S terminator. This construction, pABE7, was digested with *Kpn*I, and the 2.4 kb "patatin promoter-SBE intron i- 35S terminator" *Kpn*I fragment was isolated and inserted in the *Kpn*I site of the plant transformation vector pVictorIV Man yielding plasmid pBEA11.

## PRODUCTION OF TRANSGENIC POTATO PLANTS

### Axenic stock cultures

Shoot cultures of *Solanum tuberosum* ' Bintje ' and ' Dianella ' are maintained on a substrate (LS) of a formula according to Linsmaier, E.U. and Skoog, F. (1965), *Physiol. Plant.* 18: 100-127, in addition containing 2  $\mu$ M silver thiosulphate at 25°C and 16 h light/8 h dark.

The cultures were subcultured after approximately 40 days. Leaves were then cut off the shoots and cut into nodal segments (approximately 0.8 cm) each containing one node.

### Inoculation of potato tissues

Shoots from approximately 40 days old shoot cultures (height approximately 5-6 cms) were cut into internodal segments (approximately 0.8 cm). The segments were placed into liquid LS-substrate containing the transformed *Agrobacterium tumefaciens* containing the binary vector of interest. The *Agrobacterium* were grown overnight in YMB-substrate (di-potassium hydrogen phosphate, trihydrate (0.66 g/l); magnesium sulphate, heptahydrate (0.20 g/l); sodium chloride (0.10 g/l); mannitol (10.0 g/l); and yeast extract (0.40 g/l)) containing appropriate antibiotics (corresponding to the resistance gene of the *Agrobacterium* strain) to an optical density at 660 nm (OD-660) of approximately 0.8, centrifuged and resuspended in the LS-substrate to an OD-660 of 0.5.

The segments were left in the suspension of *Agrobacterium* for 30 minutes and then the excess of bacteria were removed by blotting the segments on sterile filter paper.

#### Co-cultivation

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The shoot segments were co-cultured with bacteria for 48 hours directly on LS-substrate containing agar (8.0 g/l), 2,4-dichlorophenoxyacetic acid (2.0 mg/l) and trans-zeatin (0.5 mg/l). The substrate and also the explants were covered with sterile filter papers, and the petri dishes were placed at 25°C and 16 h light/ 8 dark.

10

#### "Washing" procedure

After the 48 h on the co-cultivation substrate the segments were transferred to containers containing liquid LS-substrate containing 800 mg/l carbenicillin. The containers were gently shaken and by this procedure the major part of the *Agrobacterium* was either washed off the segments and/or killed.

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#### Selection

After the washing procedure the segments were transferred to plates containing the LS-substrate, agar (8 g/l), trans-zeatin (1-5 mg/l), gibberellic acid (0.1 mg/l), carbenicillin (800 mg/l), and kanamycin sulphate (50-100 mg/l) or phosphinotricin (1-5 mg/l) or mannose (5 g/l) depending on the vector construction used. The segments were sub-cultured to fresh substrate each 3-4 weeks. In 3 to 4 weeks, shoots develop from the segments and the formation of new shoots continued for 3-4 months.

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#### Rooting of regenerated shoots

The regenerated shoots were transferred to rooting substrate composed of LS-substrate, agar (8 g/l) and carbenicillin (800 mg/l).

30

The transgenic genotype of the regenerated shoot were verified by testing the rooting ability on the above mentioned substrates containing kanamycin sulphate (200 mg/l), by performing NPTII assays (Radke, S. E. et al. Theor. Appl. Genet. (1988), 75: 685-694) or by performing PCR analysis according to Wang *et al* (1993, NAR 21 pp 4153-4154). Plants which were not positive in any of these assays were discarded or used as controls. Alternatively, the transgenic plants could be verified by performing a GUS assay on the co-introduced  $\beta$ -glucuronidase gene according to Hodal, L. *et al.* (Pl. Sci. (1992), 87: 115-122).

#### Transfer to soil

The newly rooted plants (height approx. 2-3 cms) were transplanted from rooting substrate to soil and placed in a growth chamber (21°C, 16 hour light 200-400uE/m<sup>2</sup>/sec). When the plants were well established they were transferred to the greenhouse, where they were grown until tubers had developed and the upper part of the plants were senescing.

#### Harvesting

The potatoes were harvested after about 3 months and then analysed.

### BRANCHING ENZYME ANALYSIS

The SBE expression in the transgenic potato lines were measured using the SBE assays described by Blennow and Johansson (Phytochemistry (1991) 30:437-444) and by standard Western procedures using antibodies directed against potato SBE.

### STARCH ANALYSIS

Starch was isolated from potato tubers and analysed for the amylose:amylopectin ratio (Hovenkamp-Hermelink et al. (1988) Potato Research 31:241-246). In addition, the chain length distribution of amylopectin was determined by analysis of isoamylase

digested starch on a Dionex HPAEC. The number of reducing ends in isoamylase digested starch was determined by the method described by N. Nelson (1944) J. Biol.Chem. 153:375-380.

5 The results revealed that there was a reduction in the level of synthesis of SBE and/or the level of activity of SBE and/or the composition of starch SBE in the transgenic plants.

### CONSTRUCTION OF SBE PROMOTER CONSTRUCT

10

An SBE promoter fragment was amplified from  $\lambda$ -SBE 3.4 using primers:

5' CCA TCG ATA CTT TAA GTG ATT TGA TGG C 3'

15

and

5' CGG GAT CCT GTT CTG ATT CTT GAT TTC C 3'

20

The PCR product was digested with *Cla*I and *Bam*HI. The resultant 1.2 kb fragment was then inserted in pVictor5a (see Figure 9) linearised with *Cla*I and *Bgl*II yielding pBEP2 (see Figure 10).

### STARCH BRANCHING ENZYME MEASUREMENTS OF POTATO TUBERS

25

Potatoes from potato plants transformed with pBEA11 were cut in small pieces and homogenised in extraction buffer (50 mM Tris-HCl pH 7.5, Sodium-dithionit (0.1 g/l), and 2 mM DTT) using a Ultra-Turax homogenizer; 1 g of Dowex xl. was added pr. 10 g of tuber. The crude homogenate was filtered through a miracloth filter and centrifuged at 4°C for 10 minutes at 24.700 g. The supernatant was used for starch  
30 branching enzyme assays.

The starch branching enzyme assays were carried out at 25 oC in a volume of 400  $\mu$ l composed of 0.1 M Na citrate buffer pH 7.0, 0.75 mg/ml amylose, 5 mg/ml bovine serum albumin and the potato extract. At 0, 15 30 and 60 minutes aliquots of 50  $\mu$ l were removed from the reaction into 20  $\mu$ l 3 N HCl. 1 ml of iodine solution was added and the decrease in absorbance at 620 nm was measured with an ELISA spectrophotometer.

The starch branching enzyme (SBE) levels in tuber extracts were measured from 24 transgenic Dianella potato plants transformed with plasmid pBEA11.

The results showed that the BEA11 transgenic lines produced tubers which have SBE levels that are only 10 % to 15 % of the SBE levels found in non transformed Dianella plants.

## SUMMATION

The above-mentioned examples relate to the isolation and sequencing of a gene for potato SBE. The examples further demonstrate that it is possible to prepare SBE intron constructs. These SBE intron constructs can be introduced into plants, such as potato plants. After introduction, a reduction in the level of synthesis of SBE and/or the level of activity of SBE and/or the composition of starch in plants can be achieved.

Without wishing to be bound by theory it is believed that the expressed sense intron nucleotide sequence according to the present invention affects enzymatic activity *via* co-suppression and/or trans-activation. Reviews of these mechanisms has been published by Finnegan and McElroy (1994 Biotechnology 12 pp 883 - 887) and Matzke and Matzke (1995 TIG 11 No. 1 pp 1 - 3). By these mechanisms, it is believed that the sense introns of the present invention reduce the level of plant enzyme activity (in particular SBE activity), which in turn for SBE activity is believed to influence the amylose:amylopectin ratio and thus the branching pattern of amylopectin.

- Thus, the present invention provides a method wherein it is possible to manipulate the starch composition in plants, or tissues or cells thereof, such as potato tubers, by reducing the level of SBE activity by using sense intron sequences.

5 In summation the present invention therefore relates to the surprising use of sense intron sequences in a method to affect enzymatic activity in plants.

Other modifications of the present invention will be apparent to those skilled in the art without departing from the scope of the present invention. For example, it may  
10 be possible to use antisense promoter sequences to affect enzymatic activity, such as antisense SBE promoter - such as a nucleotide sequence comprising the nucleotide sequence shown as SEQ. I.D. No. 28 or a variant, derivative or homologue thereof.

The following pages present a number of sequence listings which have been  
15 consecutively numbered from SEQ.I.D. No. 1 - SEQ.I.D. No. 29. In brief, SEQ.I.D. No. 1 - SEQ.I.D. No. 13 represent sense intron sequences (genomic DNA); SEQ.I.D. No. 14 represents the SBE promoter sequence (genomic sequence); SEQ.I.D. No. 15 - SEQ.I.D. No. 27 represent antisense intron sequences; and SEQ.  
I.D. No. 28 represents the sequence complementary to the SBE promoter sequence -  
20 i.e. the SBE promoter sequence in antisense orientation. The full genomic nucleotide sequence for SBE including the promoter, exons and introns is shown as SEQ. I.D. No. 29 (see Figures 3 and 8 which highlight particular gene features).

**-SEQUENCE INFORMATION**

SEQ.I.D. No. 1

Intron 1 sequence (1167 bp).

GTAATTTTACTAATTTTCATGTTAATTTCAATTATTTTATAGCCTTTGCATTTTCATTTTCCAATATATCT  
GGATCATCTCCTTAGTTTTTTATTTTATTTTATAATATCAAATATGGAAGAAAAATGACACTTGTAG  
AGCCATATGTAAGTATCATGTGACAAATTTGCAAGGTGGTTGAGTGTATAAAATTCAAAAATTGAGAGA  
TGGAGGGGGGGTGGGGGBARAGACAATATTTAGAAAGAGTGTCTAGGAGGTTATGGAGGACACGGATG  
AGGGGTAGAAGGTTAGTTAGGTATTTGAGTGTGTCTGGCTTATCCTTTCATACTAGTAGTCGTGGAAT  
TATTTGGGTAGTTTCTTGTTTTGTTATTTGATCTTTGTTATTCTATTTTCTGTTTCTTGTACTTCGATT  
ATTGTATTATATATCTTGTCTGATGTTATTGTTTCTCGGTAAGAATGCTCTAGCATGCTTTCCTTTAGTGT  
TTTATCATGCCTTCTTTATATTGCGGTTGCTTTGAAATGCTTTTACTTTAGCCGAGGGTCTATTAGAAA  
CAATCTCTCTATCTCGTAAGGTAGGGGTAAAGTCTCACCACACTCCACTTGTGGGATTACATTGTGTT  
TGTTGTTGTAAATCAATTATGTATACATAATAAGTGGATTTTTTACAACACAAATACATGGTCAAGGGC  
AAAGTTCTGAACACATAAAGGGTTCATTATATGTCCAGGGATATGATAAAAAATTGTTTCTTTGTGAAAG  
TTATATAAGATTTGTTATGGCTTTTGCTGGAAACATAATAAGTTATAATGCTGAGATAGCTACTGAAGT  
TTGTTTTTTCTAGCCTTTTAAATGTACCAATAATAGATTCCGTATCGAACGAGTATGTTTTGATTACCT  
GGTCATGATGTTTCTATTTTACATTTTTTTGGTGTGAACTGCAATTGAAAATGTTGTATCCTATGA  
GACGGATAGTTGAGAATGTGTTCTTTGTATGGACCTTGAGAAGCTCAAACGCTACTCCAATAATTTCTA  
TGAATTCAAATTCAGTTTATGGCTACCAGTCAGTCCAGAAATTAGGATATGCTGCATATACTTGTTCAA  
TTATACTGTAAAATTTCTTAAGTTCTCAAGATATCCATGTAAACCTCGAGAATTTCTTTTGACAG

SEQ.I.D. No. 2

Intron 2 sequence (321 bp).

GTATGTTTGATAATTTATATGGTTGCATGGATAGTATATAAATAGTTGGAAATCTTCTGGACTGGTGTCT  
CATGGCATATTTGATCTGTGCACCGTGTGGAGATGTCAAACATGTGTTACTTCGTTCCGCCAATTTATA  
ATACCTTAACCTTGGGAAAGACAGCTCTTTACTCCTGTGGGCATTTGTTATTTGAATTACAATCTTTATG  
AGCATGGTGTTTTACATTATCAACTTCTTTTCATGTGGTATATAACAGTTTTTTAGCTCCGTAAATACCT  
TTCTTCTTTTGATATAAACTAACTGTGGTGCATTGCTTGCBKKK

SEQ.I.D. No. 3

Intron 3 sequence (504 bp).

GTAACAGCCAAAAGTTGTGCTTTAGGCAGTTTGACCTTATTTTGAAGATGAATTGTTTATACCTACTT  
TGACTTTTGCTAGAGAATTTTGCATACCGGGAGTAAGTAGTGGCTCCATTTAGGTGGCACCTGGCCATT  
TTTTTGATCTTTTAAAAAGCTGTTTGATTGGGTCTTCAAAAAGTAGACAAGGTTTTTGGAGAAAGTGAC  
ACACCCCCGGAGTGTCAAGTGGCAAAGCAAAGATTTTCACTAAGGAGATTCAAAATATAAAAAAGTATA  
GACATAAAACAGCTGAGGGGATTCAACATGTACTATACAAGCATCAAAATATAGTCTTAAAGCAATTTTG  
TAGAAATAAAGAAAGTCTTCTTCTGTTGCTTCACAATTTCTTCTATTATCATGAGTTACTCTTTCTG  
TTTCAAATAGCTTCTTAATATTAAATTCATGATACTTTTGTGAGATTTAGCAGTTTTTTCTTGTGTA  
AACTGCTCTCTTTTTTTTGCAG

SEQ.I.D. No. 4

Intron 4 sequence (146 bp).

GTAGGTCCTCGTCTACTACAAAATAGTAGTTTCCATCATCATAACAGATTTTCCTATTAAAGCATGATG  
TTGCAGCATCATTGGCTTTCTTACATGTTCTAATTGCTATTAAGGTTATGCTTCTAATTAACTCATCCA  
CAATGCAG

SEQ.I.D. No. 5

Intron 5 sequence (218 bp).

GTTTTGTTATTTCATACCTTGAAGCTGAATTTTGAACACCATCATCACAGGCATTTTCGATTCATGTTCTT  
ACTAGTCTTGTTATGTAAGACATTTTGAAATGCAAAAGTTAAAATAATTGTGTCTTTACTAATTTGGAC  
TTGATCCCATACTCTTCCCTTAACAAAATGAGTCAATTCATAAGTGCTTGAGAACTTACTACTTCAG  
CAATTAAACAG

SEQ.I.D. No. 6

Intron 6 sequence (198 bp).

GTATTTTAAATTTATTTCTACAATAAATAATTCTCAGAACAATTGTTAGATAGAATCCAAATATATAC  
GTCCTGAAAGTATAAAAGTACTTATTTTCGCCATGGGCCTTCAGAATATTGGTAGCCGCTGAATATCAT  
GATAAGTTATTTATCCAGTGACATTTTATGTTCACTCCTATTATGTCTGCTGGATACAG

SEQ.I.D. No. 7

Intron 7 sequence (208bp)

GTTTGTCTGTTTCTATTGCATTTTAAGGTTTCATATAGGTTAGCCACGGAAATCTCACTCTTTGTGAGG  
TAACCAGGGTTCTGATGGATTATTCAATTTTCTCGTTTATCATTGTTTATTCTTTTCATGCATTGTGT  
TTCTTTTTCATATCCCTCTTATTTGGAGGTAATTTTCTCATCTATTCACTTTTAGCTTCTAACCACAG

SEQ.I.D. No. 8

Intron 8 sequence (293 bp).

GTATGTCTTACATCTTTAGATATTTTGTGATAATTACAATTAGTTTGGCTTACTTGAACAAGATTCATT  
CCTCAAATGACCTGAACTGTTGAACATCAAAGGGGTTGAAACATAGAGGAAAACAACATGATGAATGT  
TTCCATTGTCTAGGGATTCTATTATGTTGCTGAGAACAAATGTCATCTTAAAAAAAACATTGTTTACT  
TTTTTGTAGTATAGAAGATTACTGTATAGAGTTTGCAAGTGTGTCTGTTTTGGAGTAATTGTGAAATGT  
TTGATGAACTTGATACAG

SEQ.I.D. No. 9

Intron 9 sequence (376 bp).

GTTCAAGTATTTTGAATCGCAGCTTGTTAAATAATCTAGTAATTTTATAGATTGCTTACTTGGAAAGCTA  
CTTGTTTCTGGGGATGATAGCTCATTTCATCTGTTCTACTTATTTTCCAACCGAATTTCTGATTTTGG  
TTTCGAGATCCAAGTATTAGATTCATTACACTTATTACCGCCTCATTCTACCACTAAGGCCTTGATG  
AGCAGCTTAAGTTGATTCTTTGAAGCTATAGTTTCAGGCTACCAATCCACAGCCTGCTATATTTGTTGG



ATACTTACCTTTTCTTTACAATGAAGTGATACTAATTGAAATGGTCTAAATCTGATATCTATATTTCTC  
CGTCTTTCCTCCCCCTCATGATGAAATGCAG

SEQ.I.D. No. 10

Intron 10 sequence (172 bp).

GTAAAATCATCTAAAGTTGAAAGTGTGGGTTTATGAAGTGCTTTAATTCTATCCAAGGACAAGTAGAA  
ACCTTTTTACCTTCCATTTCTTGATGATGGATTTCATATTATTTAATCCAATAGCTGGTCAAATTCGGT  
AATAGCTGTAAGTATTAGTTACTTCACTTTGCAG

SEQ.I.D. No. 11

Intron 11 sequence (145 bp).

GATATATGTTTTACTTATCCATGAAATTATTGCTCTGCTTGTTTTTAATGTACTGAACAAGTTTTATG  
GAGAAGTAACTGAAACAAATCATTTTCACATTGTCTAATTTAACTCTTTTTCTGATCCTCGCATGACG  
AAAACAG

SEQ.I.D. No. 12

Intron 12 sequence (242 bp).

GTAAGGATTTGCTTGAATAACTTTTGATAATAAGATAACAGATGTAGGGTACAGTTCTCTCACCAAAAA  
GAACTGTAATTGTCTCATCCATCTTTAGTTGTATAAGATATCCGACTGTCTGAGTTCGGAAGTGTTTGA  
GCCTCCTGCCCTCCCCCTGCGTTGTTTAGCTAATTCAAAAAGGAGAAAAGTGTATTGATGATCTTTG  
TCTTCATGCTGACATACAATCTGTTCTCATGACAG

SEQ.I.D. No. 13

Intron 13 sequence (797 bp).

GTACAGTTCTTGCCGTGTGACCTCCCTTTTATTGTGGTTTTGTTTCATAGTTATTTGAATSCGATAGAA  
GTTAACTATTGATTACCGCCACAATCGCCAGTTAAGTCCTCTGAACTACTAATTTGAAAGGTAGGAATA  
GCCGTAATAAGGTCTACTTTTGGCATCTTACTGTTACAAAACAAAAGGATGCCAAAAAATTTCTTCTCT  
ATCCTCTTTTTCCCTAAACCAAGTGCATGTAGCTTGCACCTGCATAAACTTAGGTAAATGATCAAAAATG  
AAGTTGATGGGAACCTTAAACCGCCCTGAAGTAAAGCTAGGAATAGTCATATAATGTCCACCTTTGGTG  
TCTGCGCTAACATCAACAACAACATACCTCGTGTAGTCCCAAAAGTGGTTTCAGGGGGAGGGTAGAGT  
GTATGCAAACTTACTCCTATCTCAGAGGTAGAGAGGATTTTTCAATAGACCCTTGGCTCAAGAAAAA  
AAGTCCAAAAGAAGTAACAGAAGTGAAAGCAACATGTGTAGCTAAAGCGACCCAACTTGTGTTGGGACT  
GAAAGTAGTTGTTGTTGTTGAAACAGTGCATGTAGATGAACACATGTCAGAAAATGGACAACACAGTTAT  
TTTGTGCAAGTCAAAAAATGTACTACTATTTCTTTGTGCAGCTTTATGTATAGAAAAGTTAAATAACT  
AATGAATTTTGCTAGCAGAAAAATAGCTTGGAGAGAAATTTTTATATTGAACTAAGCTAACTATATTC  
ATCTTTCTTTTGTCTTCTTCTCCTTGTGTTGTGAAG

SEQ.I.D. No. 14

DNA sequence of the SBE gene promoter region.

ATCATGGCCAATTACTGGTTCAAATGCATTACTTCCTTTCAGATTCTTTCGAGTTCTCAT	60
GACCGGTCCTACTACAGACGATACTAACCCGTGGAACGTGTCATCTGCTTCTTAGAACT	120
CTATGGCTATTTTCGTTAGCTTGGCGTCGGTTTGAACATAGTTTTTGTTCCTTCAAACCTCTT	180
CATTTACAGTCAAAATGTTGTATGGTTTTTGTTCCTCAATGATGTTTACAGTGTGTG	240
TTGTCATCTGTACTTTTGCCTATTACTTGTTCCTGAGTTACATGTTAAAAAGTGTATT	300
TTGCCATATTTTGTCTCTTATTATTATTATCATACATACATTATTACAAGGAAAAGACA	360
AGTACACAGATCTTAACGTTTATGTTCAATCAACTTTTGGAGGCATTGACAGGTACCACA	420
AATTTTGAGTTTATGATTAAAGTTCAATCTTAGAATATGAATTTAACATCTATTATAGATG	480
CATAAAATAGCTAATGATAGAACATTGACATTTGGCAGAGCTTAGGGTATGGTATATCC	540
AACGTTAATTTAGTAATTTTGTACGTACGTATATGAAATATTGAATTAATCACATGAA	600
CGGTGGATATTATATTATGAGTTGGCATCAGCAAAATCATTGGTGTAGTTGACTGTAGTT	660
GCAGATTTAATAATAAAATGGTAATTAACGGTCGATATTAATAAATCTCTCAATTCAGT	720
GGGATTAGAACTAGTTATTAATAAAATGTATACTTTAAGTGATTTGATGGCATATAATTT	780
AAAGTTTTTCATTTTCATGCTAAAATTGTTAATTATTGTAATGTAGACTGCGACTGGAATT	840
ATTATAGTGTAATTTATGCATTCAGTGTAATAATTAAAGTATTGAACTTGTCTGTTTTAG	900
AAAATACTTTTATACTTTAATATAGGATTTTGTTCATGCGAATTTAAATTAATCGATATTGA	960
ACACGGAATACCAAATTAATAAAGGATACACATGGCCTTCATATGAACCGTGAACCTTTG	1020
ATAACGTGGAAGTTCAAAGAAGGTAAAGTTTAAAGAATAAATGACAAATTAATTTCTTTT	1080
ATTTGGCCCACTACTAAATTTGCTTTACTTTCTAACATGTCAAGTTGTGCCCTCTTAGTT	1140
GAATGATATTCATTTTTTCATCCCATAGTTCAATTTGATTGTCATACCACCCATGATGTT	1200
CTGAAAAATGCTTGGCCATTCAAAAAGTTTATCTTAGTTTCTATGAACCTTTATAAGAAGC	1260
TTTAATTTGACATGTTATTTATATTAGATGATATAATCCATGACCCAATAGACAAGTGTA	1320
TTAATATTGTAACCTTTGTAATTGAGTGTGTCTACATCTTATTCATCTTTAAGGTCATT	1380
AAAATAAAATATTTTTTTGACATTCTAAAACCTTTAAGCAGAATAAATAGTTTATCAATTAT	1440
TAAAAACAATAACGACTTATTTATAAATCAACAAACAATTTTAGATTGCTCCACATAT	1500
TTTTCCAAATTAATGCAGAAAATGCATAATTTTATACTTGATCTTTATAGCTTATTTTT	1560
TTTAGCCTAACCAACGAATATTGTAACTCACAACCTTGATTAAAAGGGATTACAACAA	1620
GATATATATAAGTAGTGACAAATCTTGATTTTAAATATTTTAAATTTGGAGGTCAAAATTT	1680
TACCATAATCATTTGTATTTATAATTAATTTTAAATATCTTATTTATACATATCTAGTA	1740
AACTTTTAAATATACGTATATACAAAATATAAAATTATTGGCGTTTCATATTAGGTCAATA	1800
AATCCTTAACATATCTGCCTTACCACTAGGAGAAAGTAAAAAATCTTTTACCAAAAATA	1860
CATGTATTATGTATACAAAAAGTCGATTAGATTACCTAAATAGAAATTGTATAACGAGTA	1920
AGTAAGTAGAAATATAAAAAAATACAATACTAAAAAATATGTTTTACTTCAATTTCTG	1980
AAACTAATGGGGTCTGAGTGAAATATTCAGAAAGGGGAGGACTAACAAAAGGGTCATAAT	2040
GTTTTTTTATAAAAAAGCCACTAAAATGAGGAAATCAAGAATCAGAACATACAAGAAGGCA	2100
GCAGCTGAAGCAAAGTACCATAATTTAATCAATGGAAATTAATTTCAAAGTTTTATCAA	2160
ACCCATTCTG	

SEQ. I.D. No. 15

Intron 1 antisense sequence (1167 bp).

CTGTCAAAGAAATTCTCGAGGTTACATGGATATCTTGAGAACTTAAGAAATTTTACAGTATAATTGAAC  
AAGTATATGCAGCATATCCTAATTTCTGGACTGACTGGTAGCCATAAACTGAATTTGAATTCATAGAAA  
TTATTGGAGTAGCGTTTGAGCTTCTCAAGGTCCATACAAAGAACACATTCTCAACTATCCGTCTCATAG  
GATACAACATTTTCAATTGCAGTTCAACACCAAAAAAATGTAAAAATAGAAACATCATGACCAGGTAA  
TCAAAACATACTCGTTTCGATACGGAATCTATTATTGGTACATTTAAAAGGCTAGAAAAACAACTTCA  
GTAGCTATCTCAGCATTATAACTTATTATGTTTCCAGCAAAAGCCATAACAAATCTTATATAACTTTCA  
CAAAGAAACAATTTTTATCATATCCCTGGACATATAATGAACCCTTTATGTGTTTCAAGAACTTTGCCCTT  
GACCATGTATTTGTGTTGTAAAAATCCACTTATTATGTATACATAATTGATTTACACACACAAACACA  
ATGTAATCCCACAAGTGGAGTGTGGTGAGGACTTTACCCCTACCTTACGAGATAGAGAGATTGTTTCTA  
ATAGACCCTCGGCTAAAGTAAAAGCATTTCAAAGCAACGCGAATATAAAGAAGGCATGATAAAACACTA  
AAGGAAGCATGCTAGAGCATTCTTACCGAGGAACAATACTACGACAGATATATAATACAATAATCGA  
AGTACAAGAAACAGAAAATAGAATAACAAAGATCAAATAACAAAACAAGAACTACCCAAATAATTCCA  
CGACTACTAGTATGAAAGGATAAGCCAGACAACACTCAAATACCTAACTAACCTTCTACCCCTCATCCG  
TGTCCTCCATAACCTCCTAGAACACTCTTTCTAAATATTGTCTYTVCCCCCACCCCCCTCCATCTCTC  
AATTTTGAATTTTATACACTCAACCACCTTGCAAATTTGTCACATGATACTTACATATGGCTCTACAA  
GTGTCATTTTCTTCCATATTTGATATTATAAAAAATAAAAACTAAGGAGATGATCCAGATAT  
ATTGGAAGAAATGAAATGCAAGGCTAAAAATAATTGAAATTAACATGAAATTAGTAAAAATTAC

SEQ. I.D. No. 16

Intron 2 antisense sequence (321 bp).

MMMVGCAAGCAATGCACCACAGTTAGTTTATATCAAAAAGAAGAAAGGTATTAACGGAGCTAAAACTG  
TTATATACCACATGAAAGAAGTTGATAATGTGAAAACACCATGCTCATAAAGATTGTAATTCAAATAAC  
AAATGCCACAGGAGTAAAGAGCTGTCTTTCCCAAGTTAAGGTATTATAAATTGGCGGAACGAAGTAAC  
ACATGTTTGACATCTCCACACGGTGCACAGATCAAATATGCCATGAGCACCAGTCCAGAAGTTTCCAA  
CTATTTATATACTATCCATGCAACCATATAAATTATCAAACATAC

SEQ. I.D. No. 17

Intron 3 antisense sequence (504 bp).

CTGCAAAAAAAGAGAGCAGTTTACACAAGAAAAAAGTCTAAATCTCAACAAAAGTATCATGAATTTAA  
TATTAAGGAAGCTATTTTGAACAGAAAGAGTAACTCATGATAATAGAAGGAAATTTGTGAAGCAACAGAA  
GGAAGACTTTCTTTATTTCTACAAAATTGCTTTAAGACTATATTTGATGCTTGTATAGTACATGTTGAA  
TCCCTCAGCTTCTTTATGTCTATACTTTTTTATATTTTGAATCTCCTTAGTGAAAATCTTTGCTTTG  
CCACTGACACTCCGGGGGTGTGTCACTTCTCCAAAAACCTTGTCTACTTTTTTGAAGACCCAATCAAAC  
AGCTTTTTTAAAGATCAAAAAAATGGCCAGGTGCCACCTAAATGGAGCCACTACTTACTCCCCGGTATG  
CAAAATCTCTAGCAAAGTCAAAGTAGGTATAACAATTATCTTCCAAAATAAGGTCAAACCTGCCTAA  
AGCACAACCTTTTGGCTGTTAC

SEQ.I.D. No. 18

Intron 4 antisense sequence (146 bp).

CTGCATTGTGGATGAGTTAATTAGAAGCATAACCTTAATAGCAATTAGAACATGTAAGAAAGCCAATGA  
TGCTGCAACATCATGCTTTAATAGGAAATCTGTTATGATGATGGAACTACTATTTTGTAGTAGACGA  
GGACCTAC

SEQ.I.D. No. 19

Intron 5 antisense sequence (218 bp).

CTGTTTAATTGCTGAAGTAGTAAGTTCTCAAGCACTTATAGAATTGACTCATTTTGTAAAGGAAAGAG  
TATGGGATCAAGTCCAAATTAGTAAAGACACAATTATTTAACTTTTGCAATTCAAAATGTCTTACATA  
ACAAGACTAGTAAGAACATGAATCGAAATGCCTGTGATGATGGTGTTCAAAATTCAGCTTCAAGGTATG  
AATAACAAAAC

SEQ.I.D. No. 20

Intron 6 antisense sequence (198 bp).

CTGTATCCAGCAGACATAATAGGAGTGAACATAAAAAATGTCACTGGATAAATAAATTATCATGATATTC  
AGCGGCTACCAATATTCTGAAGGCCCATGGCGAAAAATAAGTACTTTTATACTTTCAGGACGTATATATT  
TGGATTCTATCTAACAATTGTTCTGAGAATTATTTAGTTGTAGAAATAAATTTAAATAC

SEQ.I.D. No. 21

Intron 7 antisense sequence (208 bp).

CTGTGGTTAGAAGCTAAAAGTGAATAGATGAGAAAAATTACCTCAAATAAGAGGGATATTGAAAAAGA  
AACACAATGCATGAAAAGAATAAACAAATGATAAACGAGAAAAATTGAATAATCCATCAGAACCCCTGGTT  
ACCTCACAAAGAGTGAGATTTTCCGTGGCTAACCTATATGAACCTTAAATGCAATAGAAACAGACAAAC

SEQ.I.D. No. 22

Intron 8 antisense sequence (293 bp).

CTGTACAAGTTCATCAAACATTTTACAATTACTCCAAAACAGACACACTTGCAAACCTCTATACAGTAAT  
CTTCTATACTACAAAAAAGTAAACAATGTTTTTTTTAAGATGACATTTGTTCTCAGCAACATAATAGAA  
ATCCCTAGACAATGGAACATTTCATCATGTTGTTTTCTCTATGTTTCAACCCCTTTGATGTTCAACAG  
TTCAGGTCATTTTGAGGAATGAATCTTGTTCAAGTAAGCCAACTAATTGTAATTATCACAAAATATCT  
AAAGATGTAAGACATAC

SEQ.I.D. No. 23

Intron 9 antisense sequence (376 bp).

CTGCATTTTCATCATGAGGGGGAGGAAAGACGGAGAAATATAGATATCAGATTTAGACCATTTCAATTAG  
TATCACTTCATTGTAAAGAAAAGGTAAGTATCCAACAAATATAGCAGGCTGTGGATTGGTAGCCTGAAA  
CTATAGCTTCAAAGAATCAACTTAAGCTGCTCATCAAGGCCTTAGTGGTAGAAATGAGGCGGTAAATAAG  
TGTAATGAATCTAATACTTGGATCTCGAAACAAAAATCAGAAATTCGGTTGAAAAATAAGTAGAACAA

GATGAAATGAGCTATCATCCCCAGAACCAAGTAGACTTCCAAGTAAGCAATCTAAAAATTACTAGATTATTTAACAAGCTGCGATTCAAAATACTTGAAC

SEQ.I.D. No. 24

Intron 10 antisense sequence (172 bp).

CTGCAAAGTGAAGTAACTAATCAGTACAGCTATTACCGAATTTGACCAGCTATTGGATTAAATAATATGAAATCCATCATCAAGAAATGGAAGGTAAAAAGGTTTCTACTTGTCTTGGATAGAATTAAAGCACTTCA TAAACCCAACACTTTCAACTTTAGATGATTTTAC

SEQ.I.D. No. 25

Intron 11 antisense sequence (145 bp).

CTGTTTTTCGTCATGCGAGGATCAGAAAAAGAGTTAAATTAGACAATGTGAAATGATTTGTTTCAGTTACTTCTCCATAAACTTGTTTCAGTACATTAAAAACAAGCAGAGCAATAATTTTCATGGATAAGTAAACA TATATAC

SEQ.I.D. No. 26

Intron 12 antisense sequence (242 bp).

CTGTTCATGAGAACAGATTGTATGTCAGCATGAAGACAAAGATCATCAATAAACAGTTTTCTCCTTTTTGAATTAGCTAAACAACGCAGGGGGAGGGCAGGAGGCTCAAACACTTCCGAACCTCAGACAGTCGGATATCT TATACAATAAGATGGATGAGACAATTACAGTTCTTTTTGGTGAGAGAACTGTACCCTACATCTGTTA TCTTATTATCAAAAAGTTATTCAAGCAAATCCTTAC

SEQ.I.D. No. 27

Intron 13 antisense sequence (797 bp).

CTTCACAAACAAGGAGAAGAAGAAGCAAAAAGAAAGATGAATATAGTTAGCTTAGTTCAATATAAAAAA TTTCTCTCCAAGCTATTTTTCTGCTAGCAAAATTCATTAGTTATTTAACTTTTCTATACATAAAGCTGC ACAAAGAAATAGTAGTACATTTTTTTGACTTGCACAAAATAACTGTGTTGTCCATTTTCTGACATGTGT TCATCTACATGCACTGTTTCAACAACAACAATACTTTCAGTCCCAACAAGTTGGGTCGCTTTAGCTAC ACATGTTGCTTTCCTTCTGTTACTTCTTTTTGGACTTTTTTCTTGAGCCAAGGGTCTATTGAAAAAA TCCTCTCTACCTCTGAGATAGGAGTAAGTTTTGCATACACTCTACCCTCCCCCTGAAACCACTTTGTGG GACTACACGAGGTATGTTGTTGTTGATGTTAGCGCAGACACCAAGGTGGACATTATATGACTATTCTT AGCTTTTACTTCAGGGCGGTTTTAAGTTCCCATCAACTTCATTTTTGATCATTTACCTAAGTTTATGCAG GTGCAAGCTACATGCACTGGTTTAGGGAAAAAGAGGATAGAGAAGAATTTTTTGGCATCCTTTTGTGT TGTAACAGTAAGATGCCAAAAGTAGACCTTATTACGGCTATTCTACCTTTCAAATTAGTAGTTTCAGAG GACTTAACTGGCGATTGTGGCGGTAATCAATAGTTAACTTCTATCGCATTCAAATAACTATGAACAAAA CCACAATAAAAAGGGAGGTACACACGGCAAGAAGTGTAC

SEQ.I.D. No. 28

Antisense DNA sequence of the SBE gene promoter region.

CGAATGGGTTTTGATAAACTTTGAAATTAATTTCCATTGATTAAATTATGGTACTTTGC	60
TTCAGCTGCTGCCTTCTTGATGTTCTGATTCTTGATTTCTCATTCTTAGTGGCTTTTAA	120
TAAAAAACATTATGACCCCTTTTGTTAGTCCTCCCTTTCTGAATATTTCACTCAGACCC	180
CATTAGTTTCGAAATTGAAGTAAACATATTTTTTTAGTATTGTAGTTTTTTTATATTT	240
CTACTTACTTACTCGTTATACAATTTCTATTTAGGTAATCTAATCGACTTTTTGTATACA	300
TAATACATGTATTTTTGGTAAAGAGTTTTTACTTTCTCCTAGTGGTAAGGCAGATAG	360
TTAAGGATTTATTGACCTAATATGAACGCCAATAATTTTATATTTTGTATATACGTATAT	420
TTAAAAGTTTACTAGATATGTATAAATAAGATATTTAAAATTTAATTATAAATACAAATG	480
ATTATGGTAAAATTTTGACCTCAAATTTAAATATTTAAAATCAAGATTTGTCACTACTT	540
ATATATATCTTGTTGTAAATCCCTTTTAATCAAGTTGTGAGTTTACAAATATTCGTTGGT	600
TAGGCTAAAAAAATAAGCTATAAAGATCAAGTATAAAATTATGCATTTTCTGCATTTAA	660
TTTGAAAAATATGTTGGAGCAATCTAAAATTGTTTGTGATTATATAAATAAGTCGTTTT	720
TTGTTTTTAATAATTGATAAACTATTTATTCTGCTTAAAGTTTGTAGATGTCAAAAAATA	780
ATTTATTTTAATGACCTTAAATGATTGAATAAGATGTAGACACACTCAATTACAAAGTT	840
CAATATTAATACACTTGTCTATTGGGTCATGGATTATATCATCTAATATAAATAACATGT	900
CAAATTAAAGCTTCTTATAAAGTTCATAGGAACCTAAGATAAACTTTGTGAATGGCCAAGC	960
ATTTTTCAGAACATCATGGGTGGTATGACAATCAAATTGAACTTATGGGATGAAAAATGA	1020
ATATCATTCAACTAAGAGGGCACAACTTGACATGTTAGAAAGTAAAGCAAATTTAGTAGT	1080
GGGCCAAATAAAAGAAATTAATTTGTCAGTTTATTCTTAAACTTTACCTTCTTTGAACTT	1140
CCACGTTATCAAAGGTTACGGTTCATATGAAGGCCATGTGTATCCTTTTAAATTTTGGT	1200
ATTCCGTGTTCAATATCGATTAATTTAAATTCGCATGACAAATCCTATATTAAAGTATA	1260
AAGTATTTTCTAAAACAGACAAGTTCAATACTTTAATTTTACACTGAATGCATAAATTTA	1320
CACTATAATAATCCAGTCGAGTCTACATTACAATAATTAACAATTTTAGCATGAAATG	1380
AAAAACTTTAAATTATATGCCATCAAATCACTTAAAGTATACATTTTTTTAATAACTAGT	1440
TCTAATCCCACTTGAAATGAGAGTTATTTTAAATATCGACCGTTAATTACCATTTTATTAT	1500
TAAATCTGCAACTACAGTCAACTACACCAATGATTTTGCTGATGCCAACTCATAATATAA	1560
TATCCACCGTTCATGTGATTAATTCAATATTTTATATACGTACGTAACAAAAATTACTAA	1620
ATTAACGTTGGATATACCATACCCTAAGCTCTGCCAAATGTCAATGTTCTATCATTAGCT	1680
ATTTTTATGCATCTATAATAGATGTTAAATTCATATTTCTAAGATTGAACTTAATCATAAA	1740
CTCAAAATTTGTGGTACCTGTCAATGCCTCCAAAAGTTGATTGAACATAAACGTTAAGAT	1800
CTGTGTACTTGTCTTTTCTTGTAAATAATGTATGTATGATAATAATAAGAGAACAAA	1860
ATATGGCAAAATAAACACTTTTTTAACATGTAACCTCAAAACAAGTAATAGGCAAAAGTAC	1920
AGATGACAACACAACACTGTAAACATCATTGAGGAAAAACAAAACCATACAACATTTTGA	1980
CTGTAAATGAAGAGTTTGAAAAACAAAACCTATGTTCAAACCGACGCCAAGCTAACGAAAA	2040
TAGCCATAGAGTTCTAAGAAGCAGATGCAACAGTTCCACGGGTTAGTATCGTCTGTAGTA	2100
GGACCGGTCTAGAGAACTCGAAAGAATCTGAAAGGAAGTAATGCATTTGAACCAGTAATT	2160
GGCCATGAT	

SEQ. I.D. No. 29  
Genomic SBE gene

ATCATGGCCA	ATTACTGGTT	CAAATGCATT	ACTTCCTTTC	AGATTCTTTC	GAGTTCTCAT	60
GACCGGTCCT	ACTACAGACG	ATACTAACCC	GTGGAACGTG	TGCATCTGCT	TCTTAGAACT	120
CTATGGCTAT	TTTCGTTAGC	TTGGCGTCGG	TTTGAACATA	GTTTTTGTTT	TCAAACCTCT	180
CATTTACAGT	CAAAATGTTG	TATGGTTTTT	TTTTTCCTCA	ATGATGTTTA	CAGTGTGTG	240
TTGTCATCTG	TACTTTTGCC	TATTACTTGT	TTTGAGTTAC	ATGTTAAAAA	AGTGTATTAT	300
TTGCCATATT	TTGTTCTCTT	ATTATTATTA	TCATACATAC	ATTATTACAA	GGAAAAGACA	360
AGTACACAGA	TCTTAACGTT	TATGTTCAAT	CAACTTTTGG	AGGCATTGAC	AGGTACCACA	420
AATTTTGAGT	TTATGATTAA	GTTCAATCTT	AGAATATGAA	TTTAACATCT	ATTATAGATG	480
CATAAAAATA	GCTAATGATA	GAACATTGAC	ATTTGGCAGA	GCTTAGGGTA	TGGTATATCC	540
AACGTTAATT	TAGTAATTTT	TGTTACGTAC	GTATATGAAA	TATTGAATTA	ATCACATGAA	600
CGGTGGATAT	TATATTATGA	GTTGGCATCA	GCAAAATCAT	TGGTGTAGTT	GACTGTAGTT	660
GCAGATTTAA	TAATAAAATG	GTAATTAACG	GTCGATATTA	AAATAACTCT	CATTTCAAGT	720
GGGATTAGAA	CTAGTTATTA	AAAAAATGTA	TACTTTAAGT	GATTTGATGG	CATATAATTT	780
AAAGTTTTTC	ATTTTCATGCT	AAAATTGTTA	ATTATTGTAA	TGTAGACTGC	GACTGGAATT	840
ATTATAGTGT	AAATTTATGC	ATTCAGTGTA	AAATTAAAGT	ATTGAACCTG	TCTGTTTTAG	900
AAAATACTTT	ATACTTTAAT	ATAGGATTTT	GTCATGCGAA	TTTAAATTAA	TCGATATTGA	960
ACACGGAATA	CCAAAATTAA	AAAGGATACA	CATGGCCTTC	ATATGAACCG	TGAACCTTTG	1020
ATAACGTGGA	AGTTCAAAGA	AGGTAAAGTT	TAAGAATAAA	CTGACAAATT	AATTTCTTTT	1080
ATTTGGCCCA	CTACTAAATT	TGCTTTACTT	TCTAACATGT	CAAGTTGTGC	CCTCTTAGTT	1140
GAATGATATT	CATTTTTTCAT	CCCATAAGTT	CAATTTGATT	GTCATACCAC	CCATGATGTT	1200
CTGAAAAATG	CTTGGCCATT	CACAAAGTTT	ATCTTAGTTC	CTATGAACCT	TATAAGAAGC	1260
TTTAATTTGA	CATGTTATTT	ATATTAGATG	ATATAATCCA	TGACCCAATA	GACAAGTGTA	1320
TTAATATTGT	AACTTTGTAA	TTGAGTGTGT	CTACATCTTA	TTCAATCATT	TAAGGTCATT	1380
AAAATAAATT	ATTTTTTGAC	ATTCTAAAAC	TTTAAGCAGA	ATAAATAGTT	TATCAATTAT	1440
TAAAAACAAA	AAACGACTTA	TTTATAAATC	AACAAACAAT	TTTAGATTGC	TCCAACATAT	1500
TTTTCCAAAT	TAAATGCAGA	AAATGCATAA	TTTTATACTT	GATCTTTATA	GCTTATTTTT	1560
TTTAGCCTAA	CCAACGAATA	TTTGTAAGT	CACAACTTGA	TTAAAAGGGA	TTTACAACAA	1620
GATATATATA	AGTAGTGACA	AATCTTGATT	TTAAATATTT	TAATTTGGAG	GTCAAAATTT	1680
TACCATAATC	ATTGTATTTT	ATAATTAAAT	TTTAAATATC	TTATTTTATAC	ATATCTAGTA	1740
AACTTTTAAA	TATACGTATA	TACAAAATAT	AAAATTATTG	GCGTTTCATAT	TAGGTCAATA	1800
AATCCTTAAC	TATATCTGCC	TTACCACTAG	GAGAAAGTAA	AAAACCTCTT	ACCAAAAATA	1860
CATGTATTAT	GTATACAAAA	AGTCGATTAG	ATTACCTAAA	TAGAAATTGT	ATAACGAGTA	1920
AGTAAGTAGA	AATATAAAAA	AACTACAATA	CTAAAAAATA	TATGTTTTAC	TTCAATTTTCG	1980
AAACTAATGG	GGTCTGAGTG	AAATATTCAG	AAAGGGGAGG	ACTAACAAAA	GGGTCATAAT	2040
GTTTTTTTTAT	AAAAAGCCAC	TAAAATGAGG	AAATCAAGAA	TCAGAACATA	CAAGAAGGCA	2100
GCAGCTGAAG	CAAAGTACCA	TAATTTAATC	AATGGAAATT	AATTTCAAAG	TTTTATCAAA	2160
ACCCATTTCGA	GGATCTTTTC	CTTTCTC	ACCTAAAGTT	TCTTCAGGGG	TAATTTTTCAC	2220
TAATTTTCATG	TTAATTTCAA	TTTTTAG	CCTTTGCATT	TCATTTTCCA	ATATATCTGG	2280
ATCATCTCCT	TAGTTTTTTA	TTTTATTTTT	TATAATATCA	AATATGGAAG	AAAAATGACA	2340
CTTGTAGAGC	CATATGTAAG	TATCATGTGA	CAAATTTGCA	AGGTGGTTGA	GTGTATAAAA	2400
TTCAAAAATT	GAGAGATGGA	GGGGGGGTGG	GGGBARAGAC	AATATTTAGA	AAGAGTGTTC	2460
TAGGAGGTTA	TGGAGGACAC	GGATGAGGGG	TAGAAGGTTA	GTTAGGTATT	TGAGTGTGTT	2520

CTGGCTTATC	CTTTCATACT	AGTAGTCGTG	GAATTATTTG	GGTAGTTTCT	TGTTTTGTTA	2580
TTTGATCTTT	TTTATTCTAT	TTTCTGTTTC	TTGTACTTCG	ATTATTGTAT	TATATATCTT	2640
GTCGTAGTTA	TTGTTCCCTG	GTAAGAATGC	TCTAGCATGC	TTCCTTTAGT	GTTTTATCAT	2700
GCCTTCTTTA	TATTCGCGTT	GCTTTGAAAT	GCTTTTACTT	TAGCCGAGGG	TCTATTAGAA	2760
ACAATCTCTC	TATCTCGTAA	GGTAGGGGTA	AAGTCCTCAC	CACACTCCAC	TTGTGGGATT	2820
ACATTGTGTT	TGTTGTTGTA	AATCAATTAT	GTATACATAA	TAAGTGGATT	TTTTACAACA	2880
CAAATACATG	GTCAAGGGCA	AAGTTCTGAA	CACATAAAGG	GTTCAATATA	TGTCCAGGGA	2940
TATGATAAAA	ATTGTTTCTT	TGTGAAAGTT	ATATAAGATT	TGTTATGGCT	TTTGCTGGAA	3000
ACATAATAAG	TTATAATGCT	GAGATAGCTA	CTGAAGTTTG	TTTTTTCTAG	CCTTTTAAAT	3060
GTACCAATAA	TAGATTCCGT	ATCGAACGAG	TATGTTTTGA	TTACCTGGTC	ATGATGTTTC	3120
TATTTTTTAC	ATTTTTTTGG	TGTTGAACTG	CAATTGAAAA	TGTTGTATCC	TATGAGACGG	3180
ATAGTTGAGA	ATGTGTTCTT	TGTATGGACC	TTGAGAAGCT	CAAACGCTAC	TCCAATAATT	3240
TCTATGAATT	CAAATTCAGT	TTATGGCTAC	CAGTCAGTCC	AGAAATTAGG	ATATGCTGCA	3300
TATACTTGTT	CAATTATACT	GTAAAATTTT	TTAAGTTCTC	AAGATATCCA	TGTAACCTCG	3360
AGAATTTCTT	TGACAGGCTT	CTAGAAATAA	GATATGTTTT	CCTTCTCAAC	ATAGTACTGG	3420
ACTGAAGTTT	GGATCTCAGG	AACGGTCTTG	GGATATTTCT	TCCACCCCAA	AATCAAGAGT	3480
TAGAAAAGAT	GAAAGGGTAT	GTTTGATAAT	TTATATGGTT	GCATGGATAG	TATATAAATA	3540
GTTGGAAAAC	TTCTGGACTG	GTGCTCATGG	CATATTTGAT	CTGTGCACCG	TGTGGAGATG	3600
TCAAACATGT	GTTACTTCGT	TCCGCCAATT	TATAATACCT	TAACTTGGGA	AAGACAGCTC	3660
TTTACTCCTG	TGGGCATTTG	TTATTTGAAT	TACAATCTTT	ATGAGCATGG	TGTTTTCACA	3720
TTATCAACTT	CTTTCATGTG	GTATATAACA	GTTTTTAGCT	CCGTTAATAC	CTTCTTCTT	3780
TTTGATATAA	ACTAACTGTG	GTGCATTGCT	TGCBKKKATG	AAGCACAGTT	CAGCTATTTT	3840
CGCTGTTTTG	ACCGATGACG	ACAATTCGAC	AATGGCACCC	CTAGAGGAAG	ATGTCAAGAC	3900
TGAAAATATT	GGCCTCCTAA	ATTTGGATCC	AACTTTGGAA	CCTTATCTAG	ATCACTTCAG	3960
ACACAGAATG	AAGAGATATG	TGGATCAGAA	AATGCTCATT	GAAAAATATG	AGGGACCCCT	4020
TGAGGAATTT	GCTCAAGGTA	ACAGCCAAAA	GTTGTGCTTT	AGGCAGTTTG	ACCTTATTTT	4080
GGAAGATGAA	TTGTTTATAC	CTACTTTGAC	TTTGCTAGAG	AATTTTGCAT	ACCGGGGAGT	4140
AAGTAGTGGC	TCCATTTAGG	TGGCACCTGG	CCATTTTTTT	GATCTTTTAA	AAAGCTGTTT	4200
GATTGGGTCT	TCAAAAAAGT	AGACAAGGTT	TTTGAGAGAAG	TGACACACCC	CCGGAGTGTC	4260
AGTGGCAAAAG	CAAAGATTTT	CACTAAGGAG	ATTCAAAATA	TAAAAAAAGT	ATAGACATAA	4320
AGAAGCTGAG	GGGATTCAAC	ATGTACTATA	CAAGCATCAA	ATATAGTCTT	AAAGCAATTT	4380
TGTAGAAATA	AAGAAAGTCT	TCCTTCTGTT	GCTTCACAAT	TTCTTCTAT	TATCATGAGT	4440
TACTCTTTCT	GTTTCGAAATA	GCTTCCTTAA	TATTAATTTT	ATGATACTTT	TGTTGAGATT	4500
TAGCAGTTTT	TTCTTGCTTA	AACTGCTCTC	TTTTTTTGCA	GGTTATTTAA	AATTTGGATT	4560
CAACAGGGGA	GATGGTTTCA	TAGTCTATCG	TGAATGGGCT	CCTGCTGCTC	AGTAGGTCTT	4620
CGTCTACTAC	AAAATAGTAG	TTTCCATCAT	CATAACAGAT	TTTCTTATTA	AAGCATGATG	4680
TTGCAGCATC	ATTGGCTTTT	TTACATGTTT	TAATTGCTAT	TAAGGTTATG	CTTCTAATTA	4740
ACTCATCCAC	AATGLAGGGA	AGCAGAAGTT	ATTGGCGATT	TCAATGGATG	GAACGGTTCT	4800
AACCACATGA	TGGAGAAGGA	CCAGTTTGGT	GTTTGGAGTA	TTAGAATTCC	TGATGTTGAC	4860
AGTAAGCCAG	TCATTCCACA	CAACTCCAGA	GTTAAGTTTC	GTTTCAAACA	TGGAATGGA	4920
GTGTGGGTAG	ATCGTATCCC	TGCTTGGATA	AAGTATGCCA	CTGCAGACGC	CACAAAGTTT	4980
GCAGCACCAT	ATGATGGTGT	CTACTGGGAC	CCACCACCTT	CAGAAAGGTT	TTGTTATTCA	5040
TACCTTGAAG	CTGAATTTTG	AACACCATCA	TCACAGGCAT	TTCGATTTCAT	GTTCTTACTA	5100
GTCTTGTTAT	GTAAGACATT	TTGAAATGCA	AAAGTTAAAA	TAATTGTGTC	TTTACTAATT	5160
TGGACTTGAT	CCCATACTCT	TTCCCTTAAC	AAAATGAGTC	AATTCATATA	GTGCTTGAGA	5220



ACTTACTACT	TCAGCAATTA	AACAGGTACC	ACTTCAAATA	CCCTCGCCCT	CCCAAACCCC	5280
GAGCCCCACG	AATCTATGAA	GCACATGTCG	GCATGAGCAG	CTCTGAGCCA	CGTGTAATTT	5340
CGTATCGTGA	GTTTGCAGAT	GATGTTTTAC	CTCGGATTAA	GGCAAATAAC	TATAATACTG	5400
TCCAGTTGAT	GGCCATAATG	GAACATTCTT	ACTATGGATC	ATTTGGATAT	CATGTTACAA	5460
ACTTTTTTGC	TGTGAGCAGT	AGATATGGAA	ACCCGGAGGA	CCTAAAGTAT	CTGATAGATA	5520
AAGCACATAG	CTTGGGTTTA	CAGGTTCTGG	TGGATGTAGT	TCACAGTCAT	GCAAGCAATA	5580
ATGTCACTGA	TGGCCTCAAT	GGCTTTGATA	TTGGCCAAGG	TTCTCAAGAA	TCCTACTTTC	5640
ATGCTGGAGA	GCGAGGGTAC	CATAAGTTGT	GGGATAGCAG	GCTGTTCAAC	TATGCCAATT	5700
GGGAGGTTCT	TCGTTTCCTT	CTTTCCAAC	TGAGGTGGTG	GCTAGAAGAG	TATAACTTTG	5760
ACGGATTTTCG	ATTTGATGGA	ATAACTTCTA	TGCTGTATGT	TCATCATGGA	ATCAATATGG	5820
GATTTACAGG	AACTATAAT	GAGTATTTCA	GCGAGGCTAC	AGATGTTGAT	GCTGTGGTCT	5880
ATTTAATGTT	GGCCAATAAT	CTGATTCACA	AGATTTTCCC	AGATGCAACT	GTTATTGCCG	5940
AAGATGTTTT	TGGTATGCCG	GGCCTTGCC	GGCCTGTTTC	TGAGGGAGGA	ATTGGTTTTG	6000
TTTACCGCCT	GGCAATGGCA	ATCCCAGATA	AGTGGATAGA	TTATTTAAAG	AATAAGAATG	6060
ATGAAGATTG	GTCCATGAAG	GAAGTAACAT	CGAGTTTGAC	AAATAGGAGA	TATACAGAGA	6120
AGTGTATAGC	ATATGCGGAG	ACCCATGATC	AGGTATTTTA	AATTTATTTT	TACAACTAAA	6180
TAATTCTCAG	AACAATTGTT	AGATAGAATC	CAAATATATA	CGTCCTGAAA	GTATAAAAGT	6240
ACTTATTTTC	GCCATGGGCC	TTCAGAATAT	TGGTAGCCGC	TGAATATCAT	GATAAGTTAT	6300
TTATCCAGTG	ACATTTTTAT	GTTCACTCCT	ATTATGTCTG	CTGGATACAG	TCTATTGTTG	6360
GTGACAAGAC	CATTGCATTT	CTCCTAATGG	ACAAAGAGAT	GTATTCTGGC	ATGTCTTGCT	6420
TGACAGATGC	TTCTCCTGTT	GTTGATCGAG	GAATTGCGCT	TCACAAGGTT	TGTCTGTTTC	6480
TATTGCATTT	TAAGGTTTAT	ATAGGTTAGC	CACGGAAAAT	CTCACTCTTT	CTGAGGTAAC	6540
CAGGGTTCTG	ATGGATTATT	CAATTTTCTC	GTTTATCATT	TGTTTATTCT	TTTCATGCAT	6600
TGTGTTTCTT	TTTCAATATC	CCTCTTATTT	GGAGGTAATT	TTTCTCATCT	ATTCACTTTT	6660
AGCTTCTAAC	CACAGATGAT	CCATTTTTTC	ACAATGGCCT	TGGGAGGAGA	GGGGTACCTC	6720
AATTTTCATGG	GTAACGAGGT	ATGTCTTACA	TCTTTAGATA	TTTTGTGATA	ATTACAATTA	6780
GTTTGGCTTA	CTTGAACAAG	ATTCATTCTT	CAAAATGACC	TGAACTGTTG	AACATCAAAG	6840
GGGTTGAAAC	ATAGAGGAAA	ACAACATGAT	GAATGTTTCC	ATTGTCTAGG	GATTTCTATT	6900
ATGTTGCTGA	GAACAAATGT	CATCTTAAAA	AAAACATTGT	TTACTTTTTT	GTAGTATAGA	6960
AGATTACTGT	ATAGAGTTTG	CAAGTGTGTC	TGTTTTGGAG	TAATTGTGAA	ATGTTTGATG	7020
AACTTGTACA	GTTTGGCCAT	CCTGAGTGGA	TTGACTTCCC	TAGAGAGGGC	AATAATTGGA	7080
GTTATGACAA	ATGTAGACGC	CAGTGGAACC	TCGCGGATAG	CGAACACTTG	AGATACAAGG	7140
TTCAAGTATT	TTGAATCGCA	GCTTGTTAAA	TAATCTAGTA	ATTTTATAGT	TGCTTACTTG	7200
GAAGTCTACT	TGGTCTGCGG	GATGATAGCT	CATTTTCATCT	TGTTCTACTT	ATTTTCCAAC	7260
CGAATTTCTG	ATTTTGTGTT	CGAGATCCAA	GTATTAGATT	CATTTACACT	TATTACCGCC	7320
TCATTTCTAC	CACTAAGGCC	TTGATGAGCA	GCTTAAGTTG	ATCTTTTGAA	GCTATAGTTT	7380
CAGGCTACCA	ATCCACAGCC	TGCTATATTT	GTTGGATACT	TACCTTTTCT	TTACAATGAA	7440
GTGATACTAA	TTGAAATGGT	CTAAATCTGA	TATCTATATT	TCTCCGTCTT	TCCTCCCCCT	7500
CATGATGAAA	TGCAGTTTAT	GAATGCATTT	GATAGAGCTA	TGAATTCGCT	CGATGAAAAG	7560
TTCTCATTCC	TCGCATCAGG	AAAACAGATA	GTAAGCAGCA	TGGATGATGA	TAATAAGGTA	7620
AAATCATCTA	AAGTTGAAAG	TGTTGGGTTT	ATGAAGTGCT	TTAATTCAT	CCAAGGACAA	7680
GTAGAAACCT	TTTTACCTTC	CATTTCTTGA	TGATGGATTT	CATATTATTT	AATCCAATAG	7740
CTGGTCAAAT	TCGGTAATAG	CTGTACTGAT	TAGTTACTTC	ACTTTGCAGG	TTGTTGTGTT	7800
TGAACGTGGT	GACCTGGTAT	TTGTATTCAA	CTTCCACCCA	AAGAACACAT	ACGAAGGGTA	7860
TATATGTTTT	ACTTATCCAT	GAAATTATTG	CTCTGCTTGT	TTTTAATGTA	CTGAACAAGT	7920

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GATCCTCGCA	TGACGAAAAC	AGGTATAAAG	TGGATGTGA	CTTGCCAGGG	AAGTACAGAG	8040
TTGCACTGGA	CAGTGATGCT	TGGGAATTTG	GTGGCCATGG	AAGAGTAAGG	ATTTGCTTGA	8100
ATAACTTTTG	ATAATAAGAT	AACAGATGTA	GGGTACAGTT	CTCTACCCAA	AAAGAACTGT	8160
AATTGTCTCA	TCCATCTTTA	GTTGTATAAG	ATATCCGACT	GTCTGAGTTC	GGAAGTGTTC	8220
GAGCCTCCTG	CCCTCCCCCT	GCGTTGTTTA	GCTAATTCAA	AAAGGAGAAA	ACTGTTTATT	8280
GATGATCTTT	GTCTTCATGC	TGACATACAA	TCTGTTCTCA	TGACAGACTG	GTCATGATGT	8340
TGACCATTTC	ACATCACCAG	AAGGAATACC	TGGAGTTCCA	GAAACAAATT	TCAATGGTCG	8400
TCCAAATTCC	TTCAAAGTGC	TGTCTCCTGC	GCGAACATGT	GTGGTACAGT	TCTTGCCGTG	8460
TGACCTCCCT	TTTTATTGTG	GTTTTGTTCA	TAGTTATTTG	AATGCGATAG	AAGTTAACTA	8520
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AATAGTCATA	TAATGTCCAC	CTTTGGTGTC	TGCGCTAACA	TCAACAACAA	CATACCTCGT	8820
GTAGTCCAC	AAAGTGGTTT	CAGGGGGAGG	GTAGAGTGTA	TGCAAAACTT	ACTCCTATCT	8880
CAGAGGTAGA	GAGGATTTTT	TCAATAGACC	CTTGGCTCAA	GAAAAAAGT	CCAAAAAGAA	8940
GTAACAGAAG	TGAAAGCAAC	ATGTGTAGCT	AAAGCGACCC	AACTTGTTTG	GGACTGAAGT	9000
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TTGAACTAAG	CTAACTATAT	TCATCTTTCT	TTTTGCTTCT	TCTTCTCCTT	GTTTGTGAAG	9240
GCTTATTACA	GAGTTGATGA	ACGCATGTCA	GAAACTGAAG	ATTACCAGAC	AGACATTTGT	9300
AGTGAGCTAC	TACCAACAGC	CAATATCGAG	GAGAGTGACG	AGAAACTTAA	AGATTCGTTA	9360
TCTACAAATA	TCAGTAACAT	TGACGAACGC	ATGTCAGAAA	CTGAAGTTTA	CCAGACAGAC	9420
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TCGTTATCTA	CAAATATCAG	TAACATTGAT	CAGACTGTTG	TAGTTTCTGT	TGAGGAGAGA	9540
GACAAGGAAC	TTAAAGATTG	ACCGTCTGTA	AGCATCATTG	GTGATGTTGT	TCCAGCTGAA	9600
TGGGATGATT	CAGATGCAAA	CGTCTGGGGT	GAGGACTAGT	CAGATGATTG	ATCGACCCTT	9660
CTACCGATTG	GTGATCGCTA	TCCTTGCTCT	CTGAGAAATA	GGTGAGGCGA	AACAAAAAAT	9720
AATTTGCATG	ATAAAAAGTC	TGATTTTATG	ATCGCTATCC	TCGCTCTCTG	AGAAAGAAGC	9780
GAAACAAAGG	CGACTCCTGG	ACTCGAATCT	ATAAGATAAC	AAAGGCGACT	CCTGGGACTC	9840
GAATCTATAA	GATAACAAAG	GCAATTCCAA	GACTTGAATC	TATAAAAAAT	TTAGTTAAGA	9900
ATGATTAACG	TCCGATCCTA	ATTGGAATCG	AGGCATCTTA	CCACTCCATT	GATAATTATA	9960
TAAGTCAATA	AGTCATATAA	WAGTATTAAA	AACTAAATTG	ACTTGATCGG	TCTATCAAAA	10020
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TTTCATGTGC	AATAACAAAG	AAATGATAGG	AATTTAGAGA	TTCCAATTTT	TTTGTTGCCA	10140
CAATTAACCT	AATTACATCT	TTCATTTGCA	ATAACAAAGA	AATGATAGGA	ATTTAGAGAT	10200
CCAGTGTCAA	TACACAACCT	AGGCCAACAT	CGAAAGCATA	ACTGTAAACT	CATGCATGAA	10260
GAAATCAGTC	GTAAAAATGA	ATAAATGCCA	CATAAAAACA	AATTGCATGT	ATCATTAAATG	10320
TGACTTAACT	ACAAGTAAAA	ATAAATTTAA	CAAATGTAAC	TTAACTACAA	GTAAAAATAA	10380
ATTGCTTCTA	TCATTAACAA	ACAAACAGAA	TTAAAAAGAA	AAAAACATAC	TAAATCTTAC	10440
CGTCATTCSA	TAAAAAATAA	TACCAAATTC	ATAATGCAAG	GAAAACGAAA	CGCGTCTCTG	10500
TCGGGTATCA	ACGATGAAAT	GGACCAGTTG	GATCGACTGC	CTGCACAACG	TTAGGTATGC	10560
CAAAAAAAG	AACACGATCC	TTTGCACCCG	TTCGATGATT	ATCAGTATGT	TCACAAAAAA	10620

AACCTTAAGTT	CATCCCAAGTG	TACAACAGCC	CCAACATCTG	CCCCAAGTAA	CAAAAAACAA	10680
CCAATTTATC	TTATTCTTAT	CTGCCACAAA	ATAATCGGTT	TCACACTATT	CTCTTGTTAT	10740
ACAAAATTGA	CAAGTAGGAA	GGAGAGGAGT	CATCCAAATA	AACGGTGCAC	GTTCTTTGAG	10800
AAAAGTCTTA	TTTTTCGTAA	GATCCAATTT	CAACAAACTT	TTCTTCAAGT	CAAAATTCCT	10860
GATAGTGTAT	CTCCTCTCGA	CGACCTCTTG	CATTGAACGA	TCTCCGCTTA	TCATGAAAAG	10920
TTGCTTGGAT	AACAAGTATT	GCAAGGGGGG	GACAGTAGCT	ATTAAGTTAG	TCGGCCCAAG	10980
GAAATGGAGG	AGTGATAGTC	TCGAATATTA	TTCACCTCTT	TAGCATTACC	CGGTCTGGCT	11040
TTAAGGAGTT	ACGTCTTTTA	CGCTCGCCAA	TTTCTTTTTT	TAGAATGGTT	GGTGTCAAAA	11100
TCGCGAGTTG	TGGAAGGTTT	AAGTTACTCG	ATTCGTGATT	TTCAAGTATG	AGTGGTGAGA	11160
GAGATTCGAT	ATTTTCACGA	GGTGTATTCT	AGGTCTAGTA	GAACGAAGGG	TGTCACTAAT	11220
GAAAGTTTCA	AGAGTTCATC	ATCATCTTCT	TCTAGTAGAT	TTTCGCTTTC	AAATGAGTAT	11280
GAAAATTCTT	CCTCTTTTCT	ATTGATTTTC	TTCATTSTTT	TCTTCATTGT	TGTGGTTGTT	11340
ATTGAAAAGA	AAGAAAATTT	ATAACAGAAA	AAGATGTCAA	AAAAAAGGTA	AAATGAAAGA	11400
GTATCATATA	CTTAAAGAGT	TGCGTAGAGA	TAAGTCAAAA	GAAACAGAAT	TATAGTAATT	11460
TCAGCTAAGT	TAGAATTC					11478

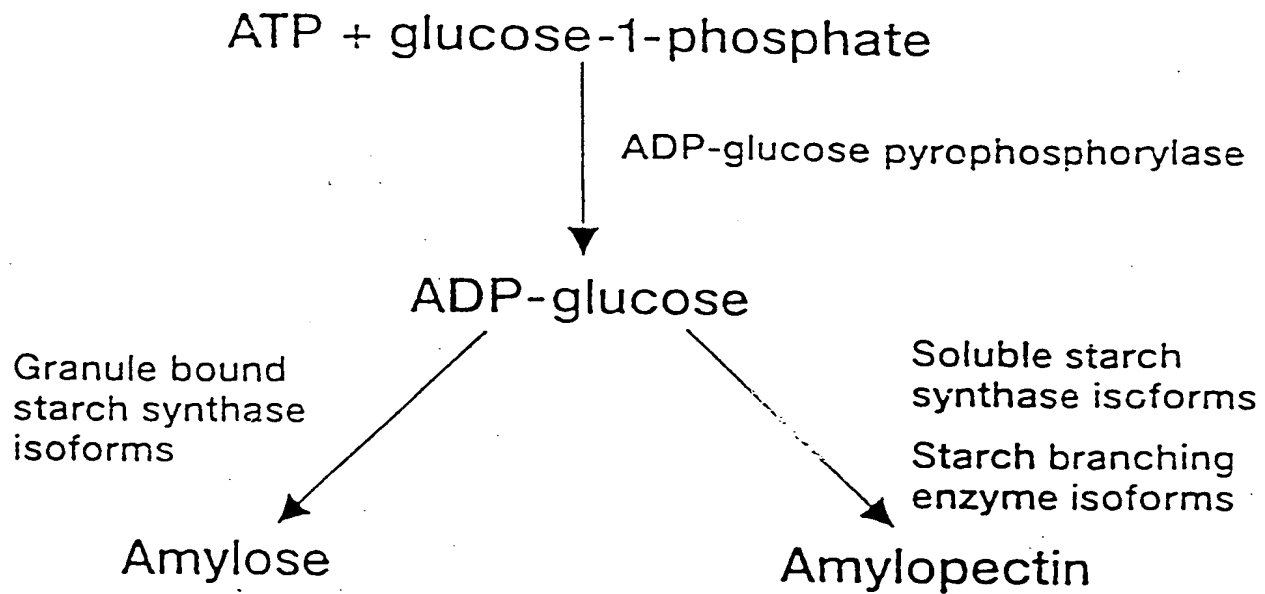
**CLAIMS**

1. A method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; and wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.
2. A method according to claim 1 wherein starch branching enzyme activity is affected and/or wherein the levels of amylopectin are affected and/or the composition of starch is changed.
3. A method of affecting enzymatic activity in a starch producing organism (or a cell, a tissue or an organ thereof) comprising expressing in the starch producing organism (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; and wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed.
4. A method according to any one of claims 1 to 3 wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence.
5. A method according to any one of the preceding claims wherein the enzymatic activity is reduced or eliminated.
6. A method according to any one of the preceding claims wherein the nucleotide sequence codes for at least substantially all of at least one intron in a sense orientation.

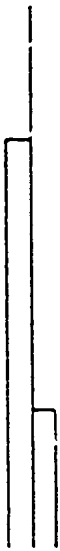
7. A method according to any one of the preceding claims wherein the nucleotide sequence codes for all of at least one intron in a sense orientation.
8. A method according to any one of the preceding claims wherein the nucleotide sequence comprises the sequence shown as any one of SEQ.I.D. No. 1 to SEQ.I.D. No. 13 or a variant, derivative or homologue thereof. including combinations thereof.
9. A method according to any one of the preceding claims wherein the nucleotide sequence is expressed by a promoter having a sequence shown as SEQ.I.D. No. 14 or a variant, derivative or homologue thereof.
10. A sense sequence comprising the nucleotide sequence as defined in claim 8 or a variant, derivative or homologue thereof.
11. A promoter having a sequence shown as SEQ.I.D. No. 14, or a variant, derivative or homologue thereof.
12. A promoter according to claim 11 in combination with a gene of interest ("GOI").
13. A construct capable of comprising or expressing the invention according to any one of claims 10 to 12.
14. A vector comprising or expressing the invention according to any one of claims 10 to 13.
15. A combination of nucleotide sequences comprising a first nucleotide sequence coding for a recombinant enzyme; and a second nucleotide sequence which corresponds to an intron in a sense orientation; wherein the intron is an intron that is associated with a genomic gene encoding an enzyme corresponding to the recombinant enzyme; and wherein the second nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

16. A cell, tissue or organ comprising or expressing the invention according to any one of claims 10 to 15.
17. A transgenic starch producing organism comprising or expressing the invention according to any one of claims 10 to 16.
18. A transgenic starch producing organism according to claim 17 wherein the organism is a plant.
19. A starch obtained from the invention according to any one of the preceding claims.
20. pBEA11 (NCIMB 40754).
21. An intron nucleotide sequence that is obtainable from  $\lambda$ -SBE 3.2 (NCIMB 40751) or  $\lambda$ -SBE 3.4 (NCIMB 40752) or a variant, derivative or homologue thereof.
22. A method of expressing a recombinant protein or enzyme in a host organism comprising expressing a nucleotide sequence coding for the recombinant protein or enzyme; and expressing a further nucleotide sequence; wherein the further nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the intron is an intron normally associated with the genomic gene encoding a protein or an enzyme corresponding to the recombinant protein or enzyme; and wherein the further nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

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Reducing end



Reducing end

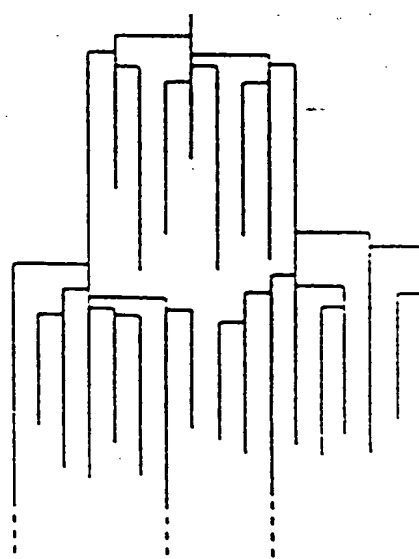


Fig 1

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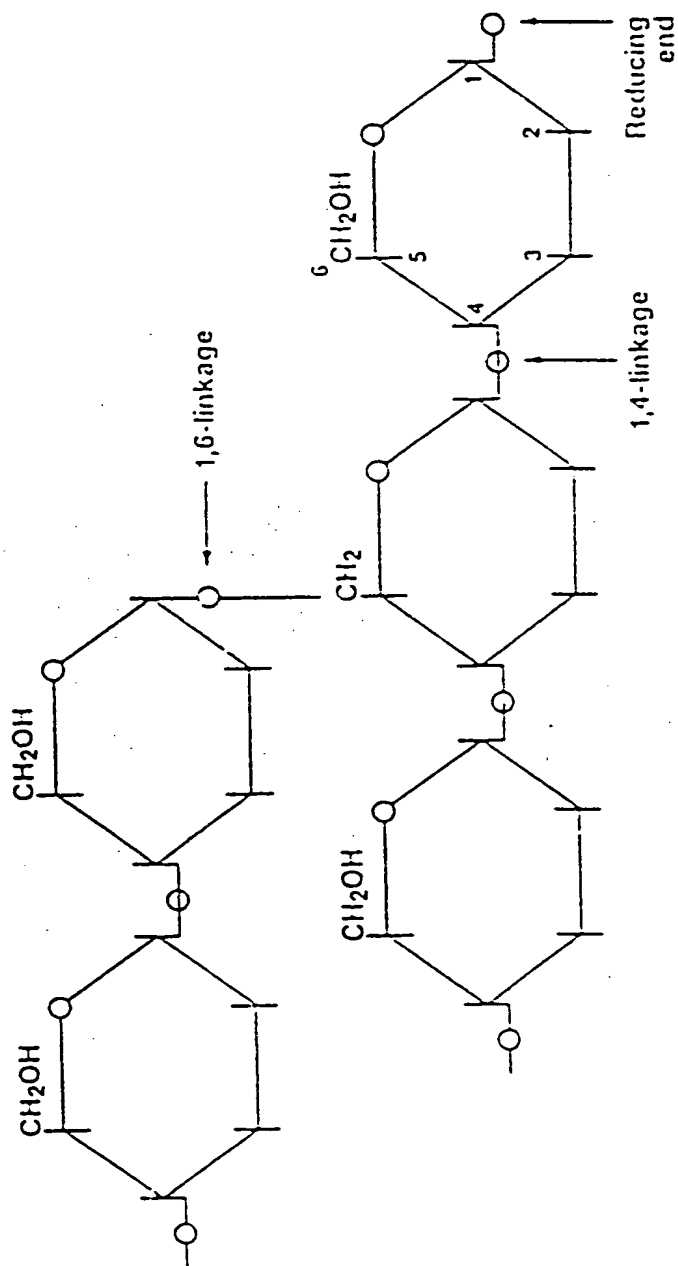


Fig 2



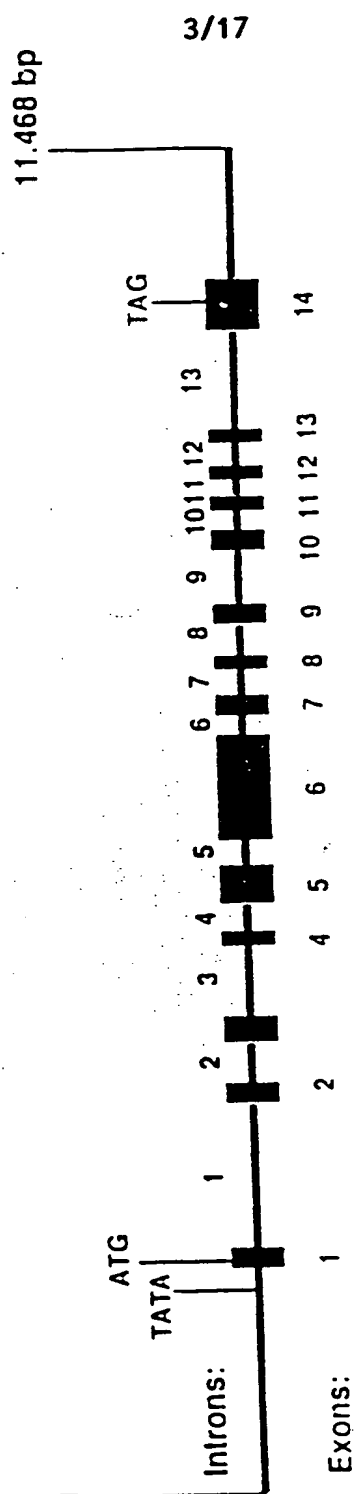


Fig 3

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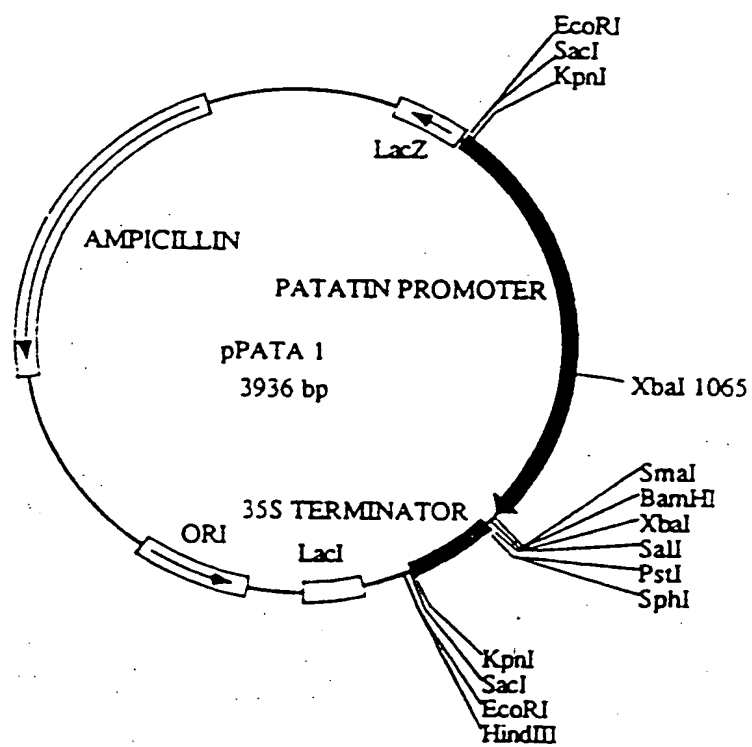


Fig 4

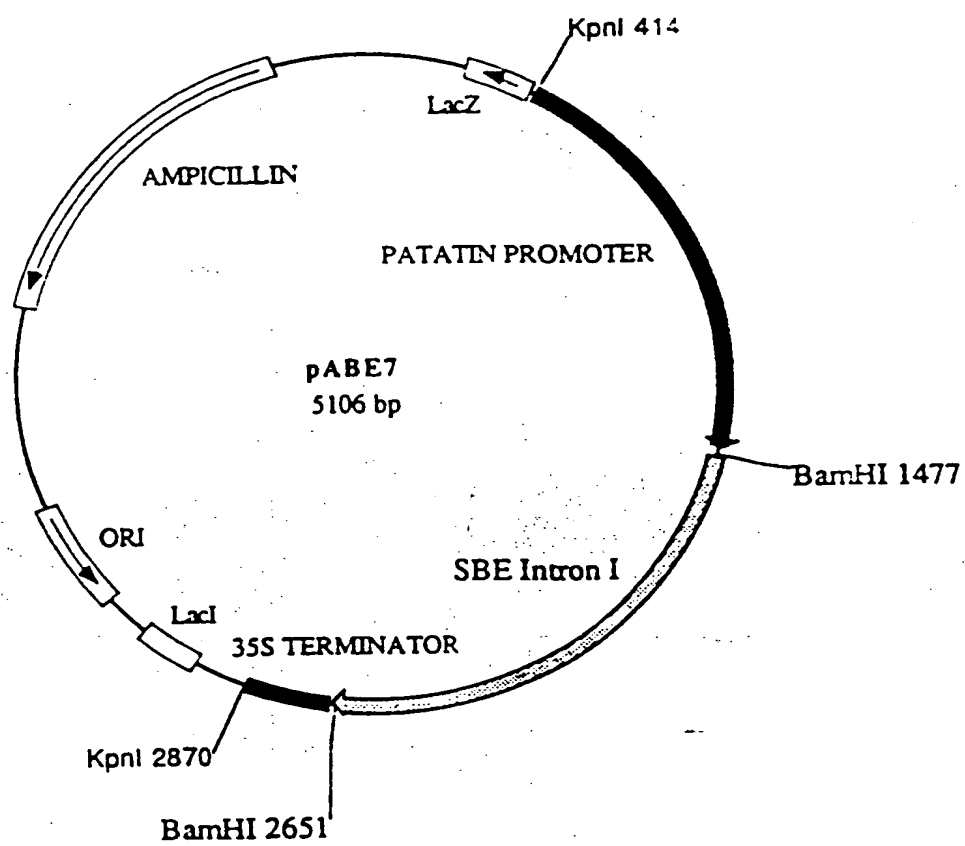


Fig 5

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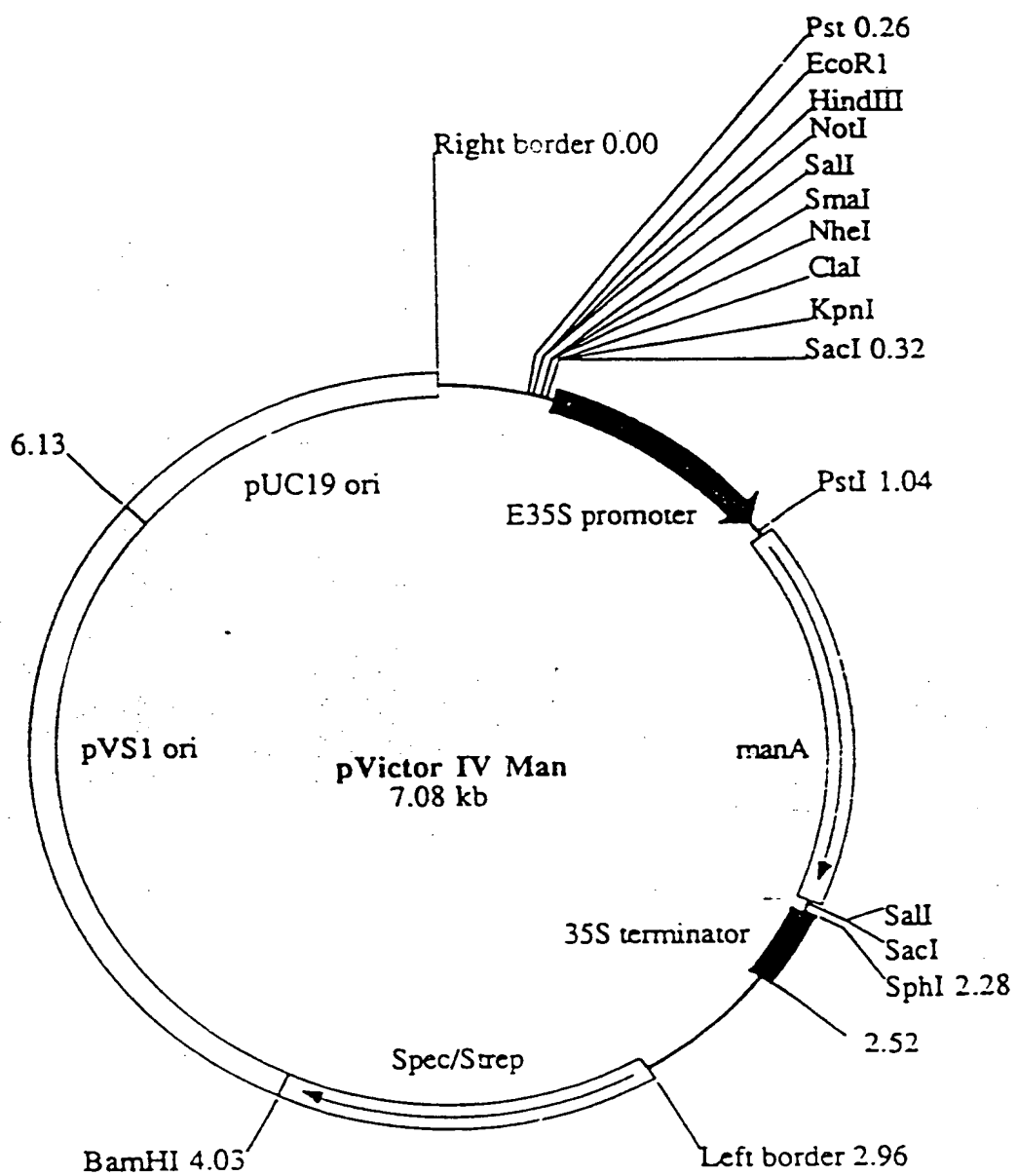


Fig 6

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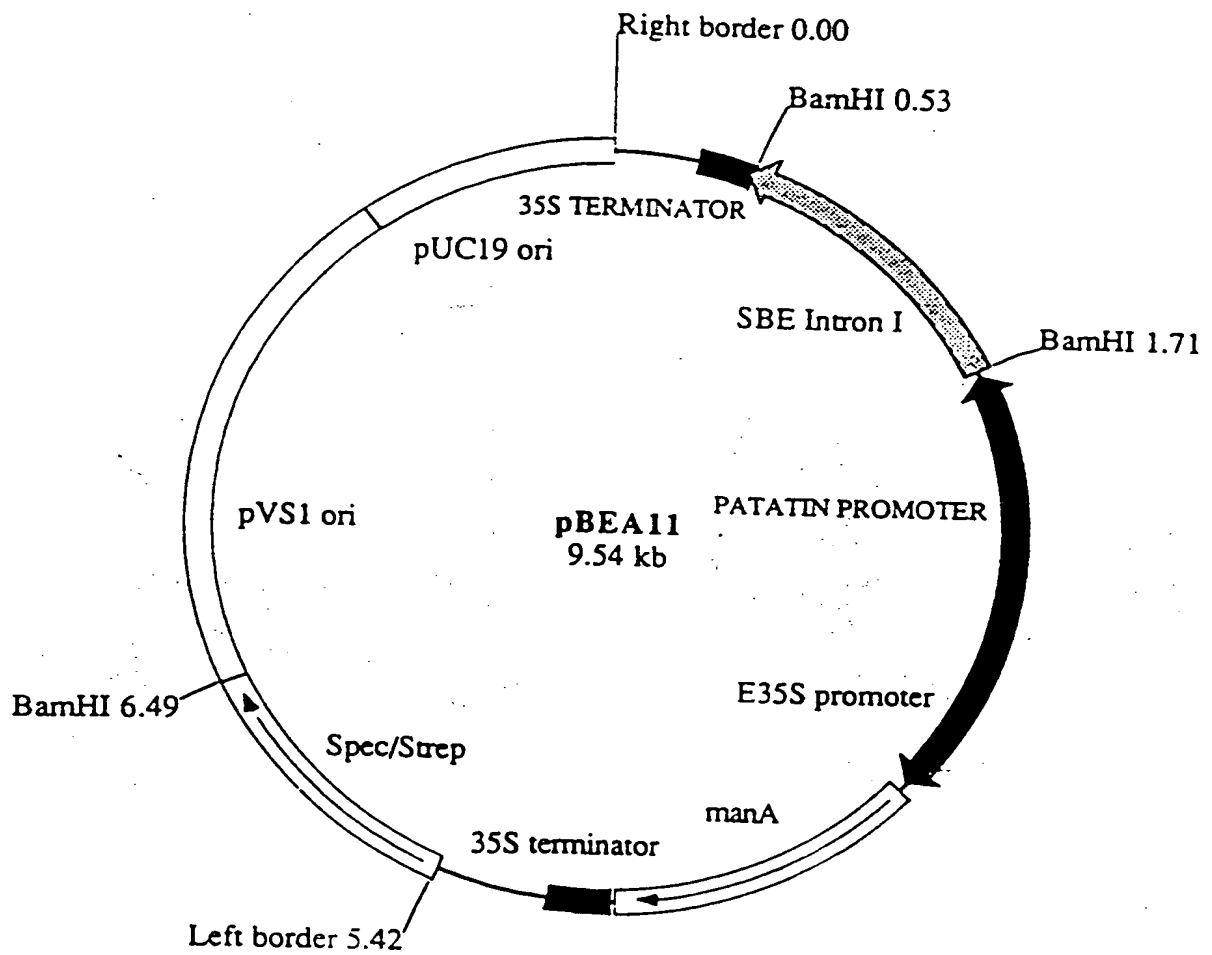


Fig 7

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10	20	30	40	50	60	
12345678901234567890123456789012345678901234567890						
ATCATGGCCAATTACTGGTTCAAATGCATTACTTCCTTTTCAGATTCTTTTCGAGTTCTCAT						60
GACCGGTCCTACTACAGACGATACTAACCCGTGGAAGTGTTCATCTGCTTCTTAGAACT						120
CTATGGCTATTTTCGTTAGCTTGGCGTCGGTTTGAACATAGTTTTTGTTCCTTCAAACCTCTT						180
CATTTACAGTCAAATGTTGTATGGTTTGTTCCTCAATGATGTTTACAGTGTGTG						240
TTGTCATCTGTACTTTTGCCTATTACTTGTTCCTGAGTTACATGTTAAAAAGTGTTCATT						300
TTGCCATATTTTGTTCCTTATTATTATTCATACATACATTATTACAAGGAAAAGACA						360
AGTACACAGATCTTAACGTTTATGTTCAATCAACTTTTGGAGGCATTGACAGGTACCACA						420
AATTTTGAGTTTATGATTAAGTTCAATCTTAGAATATGAATTTAACATCTATTATAGATG						480
CATAAAATAGCTAATGATAGAACATTGACATTTGGCAGAGCTTAGGGTATGGTATATCC						540
AACGTTAATTTAGTAATTTTGTACGTACGTATATGAAATATTGAATTAATCACATGAA						600
CGGTGGATATTATATTATGAGTTGGCATCAGCAAAATCATTGGTGTAGTTGACTGTAGTT						660
GCAGATTTAATAATAAAATGGTAATTAACGGTCGATATTAATAAATCTCATTTCAGT						720
GGGATTAGAACTAGTTATTAATAAAATGTATACTTTAAGTGATTTGATGGCATATAATTT						780
AAAGTTTTTTCATTCATGCTAAAATTGTTAATTATTGTAATGTAGACTGCGACTGGAATT						840
ATTATAGTGTAATTTTATGCATTTCAGTGTAATAAAGTATTGAACTTGTCCTGTTTTAG						900
AAAATACTTTTATACTTTAATATAGGATTTTGTTCATGCGAATTTAAATTAATCGATATTGA						960
ACACCGGAATACCAAAATTAATAAAGGATACACATGGCCTTCATATGAACCGTGAACCTTTG						1020
ATAACGTGGAAGTTCAAAGAAGGTAAAGTTAAGAATAAACTGACAAATTAATTTCTTTT						1080
ATTTGGCCCACTACTAAATTTGCTTTACTTTCTAACATGTCAAGTTGTGCCCTCTTAGTT						1140
GAATGATATTCATTTTTTCATCCATAAGTTCAATTTGATTGTCATACCACCCATGATGTT						1200
CTGAAAAATGCTTGGCCATTCAAAAGTTTATCTTAGTTCCTATGAACTTTATAAGAAGC						1260
TTTAATTTGACATGTTATTTATATTAGATGATATAATCCATGACCCAATAGACAAGTGTA						1320
TTAATATTGTAACCTTTGTAATTGAGTGTGTCTACATCTTATTCAATCATTTAAGGTCATT						1380
AAAATAAATTATTTTTTGACATTCTAAAACCTTTAAGCAGAATAAATAGTTTATCAATTAT						1440
TAAAAACAAAAACGACTTATTTATAAATCAACAAACAATTTTAGATTGCTCCAACATAT						1500

Fig 8

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10	20	30	40	50	60	
12345678901234567890123456789012345678901234567890						
TTTTCCAAATTAAATGCAGAAAATGCATAATTTTATACTTGATCTTTATAGCTTATTTT						1560
TTTAGCCTAACCAACGAATATTTGTAAACTCACAACCTTGATTAAAAGGGATTTACAACAA						1620
GATATATATAAGTAGTGACAAATCTTGATTTTAAATATTTTAAATTTGGAGGTCAAAATTT						1680
TACCATAATCATTTGTATTTATAATTAAATTTTAAATATCTTATTTATACATATCTAGTA						1740
AACTTTTAAATATACGTATATACAAAATATAAAATTATTGGCGTTCATATTAGGTCAATA						1800
AATCCTTAACTATATCTGCCTTACCACTAGGAGAAAGTAAAAAACTCTTTACCAAAAATA						1860
CATGTATTATGTATACAAAAGTCGATTAGATTACCTAAATAGAAATTGTATAACGAGTA						1920
AGTAAGTAGAAATATAAAAAAACTACAATACTAAAAAAAATATGTTTTACTTCAATTTTCG						1980
AAACTAATGGGGTCTGAGTGAAATATTCAGAAAGGGGAGGACTAACAAAAGGGTCATAAT						2040
GTTTTTTTCTTAAAAGCCACTAAAATGAGGAAATCAAGAATCAGAACATACAAGAAGGCA						2100
GCAGCTGAAGCAAAGTACCATAATTTAATCAATGGAAATTAATTTCAAAGTTTTATCAA						2160
ACCCATTCGAGGATCTTTTCCATCTTTCTCACCTAAAGTTTCTTCAGGGgtaatttttac						2220
P I R G S F P S F S P K V S S G						
taattttcatgttaattttcaattatTTTTtagcctttgcattttcattttccaatatatctgg						2280
atcatctccttagtTTTTtatttttattttttataatatcaaatatggaagaaaaatgaca						2340
cttgtagagccatatgtaagtatcatgtgacaaatttgcaagggtggttgagtgtataaaa						2400
ttcaaaaattgagagatggaggggggggtgggggbaragacaatatTTtagaaagagtgttc						2460
taggaggttatggaggacacggatgaggggtagaagggttagttaggtatttgagtgttgc						2520
ctggccttatccctttcatactagtagtcgtggaattatttgggtagtttcttgctttgcta						2580
tttgatctttgttatttctattttctgtttcttgacttcgattatttgtattatatatctt						2640
gtcgtagttattgttcctcggttaagaatgctctagcatgcttcccttttagtggtttatcat						2700
gccttcttttatattcgcggttgctttgaaatgcttttacttttagccgaggggtctattagaa						2760
acaatctctctatctcgtaaggtaggggttaaagtcctcaccacactccacttggtgggatt						2820
acattgtgtttgttggttgtaaatcaattctgtatacataataagtggattttttacaaca						2880
caaatacatgggtcaagggcaaggttctgaacacataaaggggttcatttatatgtccagggg						2940
tatgataaaaaattgtttcttttgtaaggttatataagattttgttatggcttttgctggaa						3000

Fig 8 continued.

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10	20	30	40	50	60	
12345678901234567890123456789012345678901234567890						
acataataagttataatgctgagatagctactgaagtttgttttttctagccttttaaat						3060
gtaccaataatagattccgtatcgaacgagtatgttttgattacctggcatgatgtttc						3120
tattttttacatttttttgggtgttgaactgcaattgaaaatgttgatcctatgagacgg						3180
atagttgagaatgtgttctttgtatggaccttgagaagctcaaacgctactccaataatt						3240
tctatgaattcaaattcagtttatggctaccagtcagtcagaaattaggatatgctgca						3300
tatacttgttcaattataactgtaaaatttcttaagttctcaagatatccatgtaacctcg						3360
agaatttcttttgacagGCTTCTAGAAATAAGATATGTTTTCTTCTCAACATAGTACTGG						3420
ACTGAAGTTTGGATCTCAGGAACGGTCTTGGGATATTTCTTCCACCCCAAAATCAAGAGT						3480
L K F G S Q E R S W D I S S T P K S R V						
TAGAAAAGATGAAAGGgtatgtttgataatttatatggttgcatggatagtatataaata						3540
R K D E R						
gttggaaaacttctggactggtgctcatggcatatttgatctgtgcaccgtgtggagatg						3600
tcaaacatgtgttacttcgttccgccaatttataataaccttaacttgggaaagacagctc						3660
tttactcctgtgggcatttgttatttgaattacaatctttatgagcatggtgttttcaca						3720
ttatcaacttctttcatgtggtatataacagtttttagctccgttaataacctttcttctt						3780
tttgatataaaactaactgtggtgcatttgcttgcbkkkATGAAGCACAGTTCAGCTATTTT						3840
CGCTGTTTGGACCGATGACGACAATTCGACAATGGCACCCCTAGAGGAAGATGTCAAGAC						3900
A V L T D D D N S T M A P L E E D V K T						
TGAAAATATTGGCCTCCTAAATTTGGATCCAACCTTTGGAACCTTATCTAGATCACTTCAG						3960
E N I G L L N L D P T L E P Y L D H F R						
ACACAGAATGAAGAGATATGTGGATCAGAAAATGCTCATTGAAAAATATGAGGGACCCCT						4020
H R M K R Y V D Q K M L I E K Y E G P L						
TGAGGAATTTGCTCAAGgtaacagccaaaagttgtgcttttaggcagtttgaccttatttt						4080
E E F A Q G						
ggaagatgaattgtttataacctactttgactttgctagagaattttgcataccggggagt						4140
aagtagtggtccatttaggtggcacctggccatttttttgatcttttaaaaagctgttt						4200
gattgggtcttcaaaaaagtagacaaggtttttggagaagtgcacacccccggagtgtc						4260
agtggcaaagcaaagattttcactaaggagattcaaaatataaaaaaagttagacataa						4320
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tgtagaaataaagaaagtcttccttctgttgcttcacaatttccttctattatcatgagt						4440
tactctttctgttcgaaatagcttccctaatattaaattcatgatacttttgttgagatt						4500

Fig 8 continued



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10	20	30	40	50	60	
12345678901234567890	12345678901234567890	12345678901234567890	12345678901234567890	12345678901234567890	1234567890	
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				Y L K F G F		
CAACAGGGAAGATGGTTGCATAGTCTATCGTGAATGGGCTCCTGCTGCTCAGtaggtcct						4620
N R E D G C I V Y R E W A P A A Q						
cgtctactacaaaatagtagtttccatcatcataacagattttcctattaaagcatgatg						4680
ttgcagcatcattggctttcttacatgttctaattgctattaagggttatgcttctaatta						4740
actcatccacaatgcagGGAAGCAGAAGTTATTGGCGATTTCATGGATGGAACGGTTCT						4800
	E A E V I G D F N G W N G S					
AACCACATGATGGAGAAGGACCAGTTTGGTGGTTGGAGTATTAGAATTCCTGATGTTGAC						4860
N H M M E K D Q F G V W S I R I P D V D						
AGTAAGCCAGTCATTCCACACAACCTCCAGAGTTAAGTTTCGTTTCAAACATGGTAATGGA						4920
S K P V I P H N S R V K F R F K H G N G						
GTGTGGGTAGATCGTATCCCTGCTTGGATAAAGTATGCCACTGCAGACGCCACAAAGTTT						4980
V W V D R I P A W I K Y A T A D A T K F						
GCAGCCATATGATGGTGTCTACTGGGACCCACCACCTTCAGAAAGgttttgttattca						5040
A A P Y I G V Y W D P P P S E R						
taccttgaagctgaattttgaacaccatcatcacaggcatttcgattcatgttcttacta						5100
gtcttgttatgtaagacattttgaaatgcaaaagttaaaataattgtgtctttactaatt						5160
tggacttgatcccataactctttcccttaacaaaatgagtcattctataagtgcttgaga						5220
acttactacttcagcaattaaacagGTACCACTTCAAATACCCTCGCCCTCCCAAACCCC						5280
	Y H F K Y P R P P K P R					
GAGCCCCACGAATCTATGAAGCACATGTCGGCATGAGCAGCTCTGAGCCACGTGTAAATT						5340
A P R I Y E A H V G M S S S E P R V N S						
CGTATCGTGAGTTTGCAGATGATGTTTACCTCGGATTAAGGCAAATAACTATAATACTG						5400
Y R E F A D D V L P R I K A N N Y N T V						
TCCAGTTGATGGCCATAATGGAACATTCTTACTATGGATCATTGGATATCATGTTACAA						5460
Q L M A I M E H S Y Y G S F G Y H V T N						
ACTTTTTGCTGTGAGCAGTAGATATGGAACCCGGAGGACCTAAAGTATCTGATAGATA						5520
F F A V S S R Y G N P E D L K Y L I D K						
AAGCATAGCTTGGGTTTACAGGTTCTGGTGGATGTAGTTCACAGTCATGCAAGCAATA						5580
A H S L G L Q V L V D V V H S H A S N N						
ATGTCACTGATGGCCTCAATGGCTTTGATATTGGCCAAGGTTCTCAAGAATCCTACTTTC						5640
V T D G L N G F D I G Q G S Q E S Y F H						
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A G E R G Y H K L W D S R L F N Y A N W						
GGGAGGTTCTTCGTTTCTTCTTCCAACCTTGAGGTGGTGGCTAGAAGAGTATAACTTTG						5760
E V L R F L L S N L R W W L E E Y N F D						
ACGGATTTTCGATTTGATGGAATAACTTCTATGCTGTATGTTTCATCATGGAATCAATATGG						5820
G F R F D G I T S M L Y V H H G I N M G						
GATTTACAGGAACTATAATGAGTATTTTCAGCGAGGCTACAGATGTTGATGCTGTGGTCT						5880
F T G N Y N E Y F S E A T D V D A V V Y						
ATTTAATGTTGGCCAATAATCTGATTCACAAGATTTTCCAGATGCAACTGTTATTGCCG						5940
L M L A N N L I H K I F P D A T V I A E						
AAGATGTTTCTGGTATGCCGGGCTTGGCCGGCTGTTTCTGAGGGAGGAATTGGTTTGT						6000
D V S G M P G L G R P V S E G G I G F V						

Fig 8 continued

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10	20	30	40	50	60	
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Y R L A M A I P D K W I D Y L K N K N D						
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E D W S M K E V T S S L T N R R Y T E K						
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C I A Y A E T H D Q						
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acttatttttcgccatgggccttcagaatattggtagccgctgaatatcatgataagttat						6300
ttatccagtgacatttttatgttcactcctattatgtctgctggatacagTCTATTGTTG						6360
GTGACAAGACCATTGCATTTCTCCTAATGGACAAAGAGATGTATTCTGGCATGTCTTGCT				S I V G		6420
D K T I A F L L M D K E M Y S G M S C L						
TGACAGATGCTTCTCCTGTTGTTGATCGAGGAATTGCGCTTCACAAGgtttgtctgtttc						6480
T D A S P V V D R G I A L H K						
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caggggtctgatggattattcaattttctcgtttatcatttgtttattcttttcatgcat						6600
tgtgtttctttttcaatatccctcttatttggaggttaattttctcatctattcactttc						6660
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M I H F F T M A L G G E G Y L						
AATTTTCATGGGTAACGAGgtatgtcttacatcttttagatatatttgtgataattacaatta						6780
N F M G N E						
gtttggcttacttgaacaagattcattcctcaaaatgacctgaactgttgaaatcaaag						6840
gggttgaaacatagaggaaaacaacatgatgaatgtttccattgtctagggtttctatt						6900
atgttgctgagaacaaatgtcatcttaaaaaaacattgtttactttttttagtataga						6960
agattactgtatagagtttgcaagtgtgtctgttttggagtaattgtgaaatgtttgatg						7020
aacttgtagagTTTGGCCATCCTGAGTGGATTGACTTCCCTAGAGAGGGCAATAATTGGA						7080
F G H P E W I D F P R E G N N W S						
GTTATGACAAATGTAGACGCCAGTGAACCTCGCGGATAGCGAACACTTGAGATACAAGg						7140
Y D K C R R Q W N L A D S E H L R Y K						
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gaagtctacttggttctggggatgatagctcatttcatcttggttctacttattttccaac						7260
cgaatttctgatttttgtttcgagatccaagtattagattcatttacacttattaccgcc						7320
tcatttctaccactaaggccttgatgagcagcttaagttgattctttgaagctatagt						7380
caggctaccaatccacagcctgctatatttgttgatacttacctttttcttacaatgaa						7440
gtgataactaattgaaatgggtctaaatctgatatctatatttctccgtctttcctccccct						7500

Fig 8 continued

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10	20	30	40	50	60	
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	F M N A F D R A M N S L D E K					
TTCTCATTCTCGCATCAGGAAAACAGATAGTAAGCAGCATGGATGATGATAATAAGGta						7620
F S F L A S G K Q I V S S M D D D N K						
aaatcatctaaagttgaaagtgttgggtttatgaagtgtttaattctatccaaggacaa						7680
gtagaaacctttttaccttccatttcttgatgatggatttcatattatttaaatccaatag						7740
ctggtcaaattcggtaatagctgtactgattagttacttcactttgcagGTTGTTGTGTT						7800
	V V V F					
TGAACGTGGTGACCTGGTATTTGTATTCAACTTCCACCCAAAGAACACATACGAAGGta						7860
E R G D L V F V F N F H P K N T Y E G						
tatatgttttacttatccatgaaattattgctctgcttgtttttaatgtactgaacaagt						7920
tttatggagaagtaactgaaacaaatcattttcacattgtctaatttaactcttttttct						7980
gatcctcgcatgacgaaaacagGTATAAAGTGGATGTGACTTGCCAGGGAAGTACAGAG						8040
	Y K V G C D L P G K Y R V					
TTGCACTGGACAGTGATGCTTGGGAATTTGGTGGCCATGGAAGAgtaaggatttgcttga						8100
A L D S D A W E F G G H G R						
ataacttttgataataagataacagatgtagggtacagttctctcaccaaaaagaactgt						8160
aattgtctcatccatcttttagttgtataagatatccgactgtctgagttcggaagtgtt						8220
gagcctcctgccccccccctgcgttggttagctaattcaaaaaggagaaaactgtttatt						8280
gatgatctttgtcttcatgctgacatacaatctgttctcatgacagACTGGTCATGATGT						8340
	T G H D V					
TGACCATTTACATCACCAGAAGGAATACCTGGAGTTCAGAAACAAATTTCAATGGTCG						8400
D H F T S P E G I P G V P E T N F N G R						
TCCAAATTCCTTCAAAGTGCTGTCTCTCGCGGAACATGTGTGgtacagttcttgccgtg						8460
P N S F K V L S P A R T C V						
tgacctccctttttattgtggttttgttcatagttatttgaatgcatagaagttaacta						8520
ttgattaccgccacaatcgccagttaagtctctgaactactaatttgaaaggtaggaat						8580
agccgtaataagggtctacttttggcatcttactgttacaaaacaaaaggatgccaaaaaa						8640
attcttctctatcctctttttccctaaaccagtgcattgtagcttgcacctgcataaaact						8700
aggtaaatgatcaaaaatgaagttgatgggaacttaaaaccgccctgaagtaagctagg						8760
aatagtcatataatgtccaccttttgggtgtctgcgctaacatcaacaacaacataacctgt						8820
gtagtcccacaaagtggtttcagggggagggttagagtgtatgcaaaacttactcctatct						8880
cagaggtagagaggattttttcaatagacccttggtcaagaaaaaaagtccaaaaagaa						8940
gtaacagaagtgaagcaacatgtgtagctaaagcgacccaacttggttgggactgaagt						9000

Fig 8 continued

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10	20	30	40	50	60	
123456789012345678901234567890123456789012345678901234567890						
agttgttgttgttgaacagtgcatgtagatgaacacatgtcagaaaatggacaacacag						9060
ttatatttgtgcaagtcacaaaaaatgtactactatttctttgtgcagctttatgtatagaa						9120
aagttaaataactaatgaatttttgctagcagaaaaatagcttggagagaaaattttttata						9180
ctgaactaagctaactatattcatctttctttttgtcttcttcttctccttgtttgtgaag						9240
GCTTATTACAGAGTTGATGAACGCATGTCAGAACTGAAGATTACCAGACAGACATTTGT						9300
A Y Y R V D E R M S E T E D Y Q T D I C						
AGTGAGCTACTACCAACAGCCAATATCGAGGAGAGTGACGAGAACTTAAAGATTCGTTA						9360
S E L L P T A N I E E S D E K L K D S L						
TCTACAAATATCAGTAACATTGACGAACGCATGTCAGAACTGAAGTTTACCAGACAGAC						9420
S T N I S N I D E R M S E T E V Y Q T D						
ATTTCTAGTGAGCTACTACCAACAGCCAATATTGAGGAGAGTGACGAGAACTTAAAGAT						9480
I S S E L L P T A N I E E S D E K L K D						
TCGTTATCTACAAATATCAGTAACATTGATCAGACTGTTGTAGTTTCTGTTGAGGAGAGA						9540
S L S T N I S N I D Q T V V V S V E E R						
GACAAGGAACCTAAAGATTACCGTCTGTAAAGCATCATTAGTGATGTTGTTCCAGCTGAA						9600
D K E L K D S P S V S I I S D V V P A E						
TGGGATGATTGAGATGCAACGTCTGGGGTGAGGACTAGTCAGATGATTGATCGACCCTT						9660
W D D S D A N V W G E D						
CTACCGATTGGTGATCGCTATCCTTGCTCTCTGAGAAATAGGTGAGGCGAAACAAAAAT						9720
AATTTGCATGATAAAAAGTCTGATTTTATGATCGCTATCCTCGCTCTCTGAGAAAGAAGC						9780
GAAACAAAGGCGACTCCTGGACTCGAATCTATAAGATAACAAAGGCGACTCCTGGGACTC						9840
GAATCTATAAGATAACAAAGGCAATTCGAAGACTTGAATCTATAAAAAATTTAGTTAAGA						9900
ATGATTAACGTCCGATCCTAATTCGAATCGAGGCATCTTACCACTCCATTGATAATTATA						9960
TAAGTCAATAAGTCATATAAWAGTATTAAAACTAAATTGACTTGATCGGTCTATCAAAA						10020
ATMAGATMAAATTGTGTTTCATATGTAACATTTTTTGTGTGTCACAATTAGCTTAATTACATC						10080
TTTCATGTGCAATAACAAAGAAATGATAGGAATTTAGAGATTCCAATTTTTTTGTGCGCA						10140
CAATTAACCTTAATTACATCTTTTCATTTGCAATAACAAAGAAATGATAGGAATTTAGAGAT						10200
CCAGTGTCAATACACAACCTAGGCCAACATCGAAAGCATAACTGTAACTCATGCATGAA						10260
GAAATCAGTCGTAAAAATGAATAAATGCGACATAAAAAACAAATTGCATGTATCATTAATG						10320
TGACTTAACTACAAGTAAAAATAAATTTAACAAATGTAACCTTAACTACAAGTAAAAATAA						10380
ATTGCTTCTATCATTAACAAACAAACAGAATTA AAAAGAAAAAACATACTAAATCTTAC						10440
CGTCATTGATAAAAAAAATACCAAATTCATAATGCAAGGAAAACGAAACGCGTCCTGA						10500

Fig 8 continued

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10	20	30	40	50	60	
12345678901234567890123456789012345678901234567890						
TCGGGTATCAACGATGAAATGGACCAGTTGGATCGACTGCCTGCACAACGTTAGGTATGC						10560
CAAAAAAAGAACACGATCCTTTCACCCGTTTCGATGATTATCAGTATGTTCAAAAAA						10620
AACTTAAGTTCATCCCAGTGTACAACAGCCCCAACATCTGCCCCAAGTAACAAAAACAA						10680
CCAATTTATCTTATTCTTATCTGCCACAAAATAATCGGTTTCACACTATTCTCTTGTAT						10740
ACAAAATTGACAAGTAGGAAGGAGAGGAGTCATCCAAATAAACGGTGCACGTTCTTTGAG						10800
AAAAGTCTTATTTTTTCGTAAGATCCAATTTCAACAACTTTTCTTCAAGTCAAAATTCCT						10860
GATAGTGTATCTCCTCTCGACGACCTCTTGCAATTGAACGATCTCCGCTTATCATGAAAAG						10920
TTGCTTGGATAACAAGTATTGCAAGGGGGGACAGTAGCTATTAAGTTAGTCGGCCCAAG						10980
GAAATGGAGGAGTGATAGTCTCGAATATTATTCACCTCTTTAGCATTACCCGGTCTGGCT						11040
TTAAGGAGTTACGTCTTTTACGCTCGCCAATTTCTTTTTTTAGAAATGGTTGGTGTCAAAA						11100
TCCCGAGTTGTGGAAGGTTCAAGTTACTCGATTTCGTGATTTTCAAGTATGAGTGGTGAGA						11160
GAGATTCGATATTTTCACGAGGTGTATTGAGGTCTAGTAGAACGAAGGTGTCACTAAT						11220
GAAAGTTTCAAGAGTTCATCATCATCTTCTTCTAGTAGATTTTCGCTTTCAAATGAGTAT						11280
GAAAATTCTTCCTCTTTTCTATTGATTTTCTTCATTGTTTTCTTCATTGTTGTGGTTGTT						11340
ATTGAAAAGAAAGAAAATTTATAACAGAAAAAGATGTCAAAAAAAGGTAAAATGAAAGA						11400
GTATCATATACTTAAAGAGTTGCGTAGAGATAAGTCAAAAGAAACAGAATTATAGTAATT						11460
TCAGCTAAGTTAGAATTC						11478

Fig 8 continued

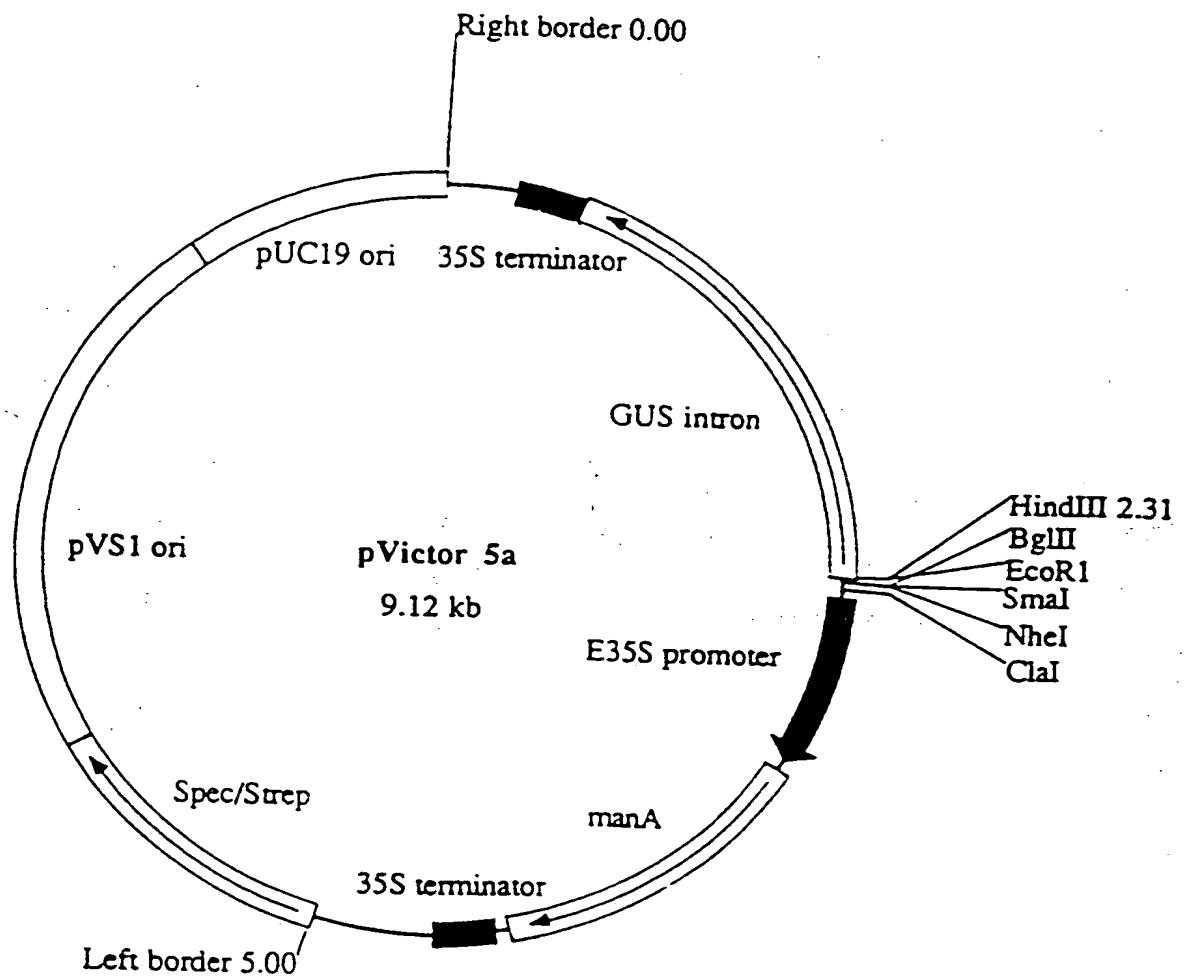


Fig 9

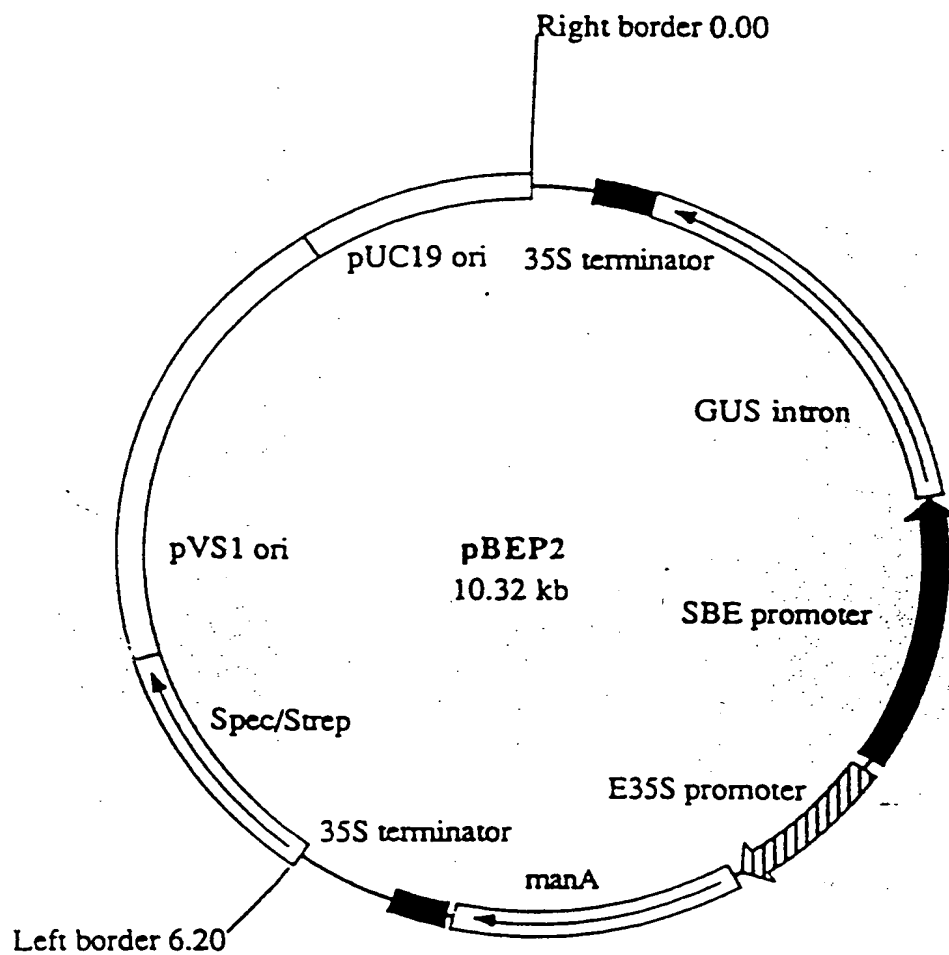


Fig 10

PLT/EP 96 / 03053

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>19</u> , lines <u>18 to 27</u>	
B. IDENTIFICATION OF DEPOSIT <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution  The National Collections of Industrial and Marine Bacteria Limited (NCIMB)	
Address of depositary institution (including postal code and country)  23 St. Machar Drive Aberdeen Scotland AB2 1RY United Kingdom	
Date of deposit  13 July 1995	Accession Number  NCIMB 40754, NCIMB 40751, NCIMB 40752
C. ADDITIONAL INDICATIONS (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
In respect of those designations in which a European patent is sought, and any other designated state having equivalent legislation, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC).	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer  L.R. Pether	Authorized officer



BUDAPEST TREATY ON THE INTERNATIONAL  
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS  
FOR THE PURPOSES OF PATENT PROCEDURE

PCT/EP 96 / 03053

Danisco Biotechnology  
Jægersgade 1  
P.O. Box 17  
DK-1001 Copenhagen K  
Denmark

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT  
issued pursuant to Rule 7.1 by the  
INTERNATIONAL DEPOSITARY AUTHORITY  
identified at the bottom of this page

<b>I. IDENTIFICATION OF THE MICROORGANISM</b>	
Identification reference given by the DEPOSITOR:  Escherichia coli DH5 $\alpha$ -pBEA11	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:  NCIMB 40754
<b>II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION</b>	
The microorganism identified under I above was accompanied by:  <input type="checkbox"/> a scientific description  <input checked="" type="checkbox"/> a proposed taxonomic designation  (Mark with a cross where applicable)	
<b>III. RECEIPT AND ACCEPTANCE</b>	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 13 July 1995 (date of the original deposit) <sup>1</sup>	
<b>IV. RECEIPT OF REQUEST FOR CONVERSION</b>	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)	
<b>V. INTERNATIONAL DEPOSITARY AUTHORITY</b>	
Name:  <b>NCIMB Ltd</b> 23 St Machar Drive Aberdeen Scotland UK AB2 1RY	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):  Date: 21 July 1995 <i>Terence Dando</i>

<sup>1</sup> Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL  
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS  
FOR THE PURPOSES OF PATENT PROCEDURE

Danisco Biotechnology  
Langebrogade 1  
P O Box 17  
DK-1001 Copenhagen K  
Denmark

PCT/EP96/03053

INTERNATIONAL FORM

VIABILITY STATEMENT  
issued pursuant to Rule 10.2 by the  
INTERNATIONAL DEPOSITARY AUTHORITY  
identified on the following page

NAME AND ADDRESS OF THE PARTY  
TO WHOM THE VIABILITY STATEMENT  
IS ISSUED

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: As above Address:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 40754 Date of the deposit or of the transfer: 13 July 1995
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 13 July 1995 <sup>2</sup> . On that date, the said microorganism was <input checked="checked" type="checkbox"/> <sup>3</sup> viable <input type="checkbox"/> <sup>3</sup> no longer viable	

<sup>1</sup> Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

<sup>2</sup> In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

<sup>3</sup> Mark with a cross the applicable box.

A. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED<sup>4</sup>

## V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:

**NCIMB Ltd**

Address:

23 St Machar Drive  
Aberdeen Scotland  
AB9 8DVSignature(s) of person(s) having the power  
to represent the International Depositary  
Authority or of authorized official(s):

Date:

*Terence Dando*  
21 July 1995<sup>4</sup> Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL  
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS  
FOR THE PURPOSES OF PATENT PROCEDURE

DR A Buchter-Larsen  
Danisco Biotechnology  
Ingebrogade 1  
P O Box 17  
DK-1001 Copenhagen  
Denmark

INTERNATIONAL FORM

PCT/EP 96 / 03053

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INTERNATIONAL DEPOSITARY AUTHORITY  
identified at the bottom of this pag

<b>I. IDENTIFICATION OF THE MICROORGANISM</b>	
Identification reference given by the DEPOSITOR:  Bacteriophage $\lambda$ EMBL3 SP6/T7 $\lambda$ SEED.2	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:  NCIMB 40751
<b>II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION</b>	
The microorganism identified under I above was accompanied by:  <input type="checkbox"/> a scientific description  <input checked="" type="checkbox"/> a proposed taxonomic designation  (Mark with a cross where applicable)	
<b>III. RECEIPT AND ACCEPTANCE</b>	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 13 July 1995 (date of the original deposit) <sup>1</sup>	
<b>IV. RECEIPT OF REQUEST FOR CONVERSION</b>	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)	
<b>V. INTERNATIONAL DEPOSITARY AUTHORITY</b>	
Name: <b>NCIMB Ltd</b> <b>23 St Machar Drive</b> <b>Aberdeen Scotland</b> Address: <b>UK AB2 1CV</b>	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):  Date: 20 July 1995

<sup>1</sup> Where Rule 6.4(d) applies, such date is the date on which the status of international depositary  
authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL  
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS  
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Dr A Buchter-Larsen  
Danisco Biotechnology  
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DK-1001 Copenhagen K  
Denmark

INTERNATIONAL FORM

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INTERNATIONAL DEPOSITARY AUTHORITY  
identified on the following page

NAME AND ADDRESS OF THE PARTY  
TO WHOM THE VIABILITY STATEMENT  
IS ISSUED

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: As above  Address:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 40751  Date of the deposit or of the transfer:  13 July 1995
III. VIABILITY STATEMENT	
<p>The viability of the microorganism identified under II above was tested on 19 July 1995<sup>1</sup>. On that date, the said microorganism was</p> <p><input checked="" type="checkbox"/><sup>3</sup> viable</p> <p><input type="checkbox"/><sup>3</sup> no longer viable</p>	

<sup>1</sup> Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

<sup>2</sup> In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

<sup>3</sup> Mark with a cross the applicable box.

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED<sup>4</sup>

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:

Address:

**NCIMB Ltd**  
23 St Machar Drive  
Aberdeen Scotland  
UK AB2 1RY

Signature(s) of person(s) having the power  
to represent the International Depositary  
Authority or of authorized official(s):

Date:

20 July 1995

<sup>4</sup> Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL  
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INTERNATIONAL FORM

**PCT/EP 96 / 03053**

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Issued pursuant to Rule 7.1 by the  
INTERNATIONAL DEPOSITARY AUTHORITY  
identified at the bottom of this page

NAME AND ADDRESS  
OF DEPOSITOR

<b>I. IDENTIFICATION OF THE MICROORGANISM</b>	
Identification reference given by the DEPOSITOR:  Bacteriophage $\lambda$ EMBL3 SP6/T7 $\lambda$ SBZ3.4	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:  NCIMB 40752
<b>II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION</b>	
The microorganism identified under I above was accompanied by:	
<input type="checkbox"/> a scientific description	
<input checked="" type="checkbox"/> a proposed taxonomic designation	
(Mark with a cross where applicable)	
<b>III. RECEIPT AND ACCEPTANCE</b>	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 13 July 1995 (date of the original deposit) <sup>1</sup>	
<b>IV. RECEIPT OF REQUEST FOR CONVERSION</b>	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)	
<b>V. INTERNATIONAL DEPOSITARY AUTHORITY</b>	
Name:  <b>NCIMB Ltd</b> 23 St Machar Drive Aberdeen Scotland Address: UK AP2	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):  Date: 20 July 1995

<sup>1</sup> Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

PCT/EP96/03053

BUDAPEST TREATY ON THE INTERNATIONAL  
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I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: As above  Address:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 40752  Date of the deposit or of the transfer:  13 July 1995
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 19 July 1995 <sup>1</sup>  <input checked="" type="checkbox"/> <sup>3</sup> viable  <input type="checkbox"/> <sup>3</sup> no longer viable	

<sup>1</sup> Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

<sup>2</sup> In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

<sup>3</sup> Mark with a cross the applicable box.



IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED<sup>4</sup>

## V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:

**NCIMB Ltd**

Address:

23 St Machar Drive  
Aberdeen Scotland  
AB9 1RYSignature(s) of person(s) having the power  
to represent the International Depositary  
Authority or of authorized official(s):

Date:

20 July 1995

<sup>4</sup> Fill in if the information has been requested and if the results of the test were negative.